THE SPACE SCIENCES RESEARCH CENTER

SEMI-ANNUAL STATUS REPORT

NGL 26-004-021 S 5,6&7a

X.J. Musacchia, Ph.D.

RESEARCHING
THE ENVIRONMENTAL
SCIENCES

UNIVERSITY OF MISSOURI-COLUMBIA
COLUMBIA, MISSOURI

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University of Missouri
Space Sciences Research Center
Columbia, Missouri 65201

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PREFACE

The following is a resumé of research activities supported by NASA Grant NGL 26-004-021, S 5, 6 and 7a. These projects were started during earlier periods of this grant and are currently in progress. Research results for these projects are reported in SECTION I. Reprints of published results of completed projects are provided in SECTION II.

The overall program encompasses several areas: physiology of depressed metabolism, radioresistance in depressed metabolic states, comparative aspects of depressed metabolism, and gastrointestinal responses to ionizing radiation. Projects inclusive of these areas are done in my laboratories in the Space Sciences Research Center, (SSRC) University of Missouri, Columbia. Obviously these projects are often interdisciplinary and they involve a variety of individuals each with his own expertise. Dr. Wynn Volkert, (radio-biology and physical chemistry) has actively collaborated in many of the radiation projects and in physiological studies of depressed metabolism. Dr. Gary Anderson (physiology) has participated in much of the research centered on physiologic aspects of helium-cold hypothermia. Dr. Wesley Platner (physiology) and Dr. B. C. Patnayak (animal sciences) have collaborated on a long term project concerned with lipid metabolism and hibernation. More recently, Dr. Alexander Kenny (biochemistry and pharmacology) has become involved in pilot studies of calcitonin activity and calcium metabolism in metabolically depressed animals. These individuals (and others mentioned in

earlier reports) have been involved in collaborative intramural re-
search where our laboratory has served as the primary site for
research.

Two active multidisciplinary projects are underway with
colleagues outside the University of Missouri. One program in
intermediary metabolism involves a series of collaborative projects
with Dr. Cecil Entenman, Institute for Lipid Research, Berkeley,
California. Another program was initiated with Dr. Jiro Oyama
at the NASA Ames Research Center, California. The current project
is concerned with the effects of increased G forces on growth and
behavior of hamsters. This is intended as a prelude to more inten-
sive studies of increased G forces on the normothermic and hypothermic
hamster. Dr. Oyama is working with hamsters from the closed colony at
the University of Missouri. These projects therefore involve co-
operation between two extramural laboratories and mine in the SSRC.
It is hoped that these cooperative ventures will form the base for more
extensive efforts in the future.

Since my last status report, the NASA grantees concerned with
depressed metabolism held a second meeting in July, 1972, at
Berkeley (the first meeting was at the NASA Installation at Wallops
Station in the Fall, 1971). At the July meeting we reviewed the
prospects for extra-terrestrial experiments utilizing depressed me-
tabolic states. It was the concensus of the individuals at that
meeting that the golden hamster, Mesocricetus auratus , appeared to
be the best subject for depressed metabolism studies.

To date, two other investigators have begun to use this species for preliminary investigations, Dr. Jiro Oyama, (NASA Ames Research Center) and Dr. J. P. Jordon (Colorado State University, Fort Collins, Colorado). In addition, hamsters from our colony have been shipped to Dr. Emily Holton (NASA, Wallops Island) for some initial hematologic studies. I am confident that use of the same animal species, and in particular the same laboratory strain, for a wide variety of studies in the same area of interest ("depressed metabolism") should provide more substantial results. Consistency and reduced variability should assist us in reaching our goal to participate in bioscience programs utilizing depressed metabolic states.

The personnel affiliated with me and working in my laboratory are identified with each project herein reported. In addition, there are two new people: Dr. George Tempel, a post-doctoral, who came from the Physiology Department, University of Indiana and Jane Utsler, a research technician. Dr. Tempel's training was in environmental physiology and during his first month in this laboratory he has initiated two projects: (A) the effects of argon in oxygen uptake in rats and hamsters and (B) renal function in helium-cold hypothermic hamsters. Dr. Tempel fills my postdoctoral position which was vacated when Dr. Gary Anderson joined the staff at Georgia Tech University. Dr. Anderson was with us for two years and his interests continue in depressed metabolism. Using animals and techniques from this laboratory he has started a research program in his new location.

His contributions to our program are readily witnessed in the series of papers published from our laboratory during the last two years.

Jane Utsler replaced one of our technicians, Mollie Jacobs, who entered Veterinary School in September. Mrs. Utsler has a B.S. from Knox College (Galesburg, Illinois) where she majored in physics. She worked for a year for a neurophysiologist and is well trained for the work requirements in our laboratory.

In brief, the laboratory is composed of two full time research technicians, three doctoral students, one postdoctoral, one associate investigator, several collaborating colleagues and myself. The supporting staff of animal caretakers, electronics and machine shop personnel, typing and stenographic personnel all remain unchanged in the Space Sciences Research Center.

Grant funds are being spent and allocated as they are received. Several points of confusion between the University's business office and the SSRC administrative office appear to have been clarified. A grant application for continuation was submitted several weeks ago.

For convenience to the reader, this report is separated into two Sections. SECTION I contains brief reviews of projects underway and SECTION II contains reprints and abstracts of papers published or given before various society meetings.

FACTORS PERTINENT TO INCREASED SURVIVAL IN HELIUM-COLD HYPOTHERMIC HAMSTERS

X. J. Musacchia, Garth Resch, Wynn Volkert, and Gary Anderson

INTRODUCTION

This report reviews the progress of two investigations identified in the January Semi-Annual Status Report: "3. Bioenergetics of Helium-Cold Hypothermia" and "5. Halothane-Helox Hypothermia: An Improved Method of Induction". A report by Musacchia, et al., 1972, was given before a joint meeting of the American Physiological Society and the Division of Comparative Physiology and Biochemistry of the American Society of Zoologists, August 29, 1972. An abstract is submitted under Section II of this Semi-Annual Status Report.

Two primary objectives of our research have been to reduce the induction time for hypothermia and to prolong the survival time of an animal in a depressed metabolic state. Previous studies have shown that induction time can be significantly shortened by previous exposure to heat, about 34°C, for one week or more (Musacchia, 1972) and with depiliation by fur clipping (Prewitt, Anderson and Musacchia, 1972). In both experimental procedures the temperature regulating system is modified so that effective heat production is decreased

substantially. Hamsters that are physiologically adjusted to produce less heat in order to maintain thermal balance or that are physically deprived of an insulating cover are speeded into hypothermia because they have lost major components essential to heat production or heat conservation.

In addition, we learned from the work of Prewitt et al. (loc. cit.) that there is a positive correlation between lengthy induction times and lowered plasma glucose levels; with long induction times there are marked reductions in plasma glucose.

The objectives of the present experiments were (A) to explore a new method for shortening induction time and (B) to determine how the metabolism of liver glycogen and plasma glucose levels are related to induction and survival in hypothermia.

MATERIALS AND METHODS

Induction of hypothermia: A standard operating procedure has been to expose hamsters to a mixture of 80% helium and 20% oxygen (helox) at 0°C until the animal becomes hypothermic with body temperatures about T_{re} 7°C. This has been referred to as the helium-cold method for induction of hypothermia. The steps are outlined in Figure 1. A variety of modifications of these procedures have been done and many have been reported in previous Semi-Annual Status Reports.

FIGURE 1

STEPS IN HELIUM COLD HYPOTHERMIA

1. Animals in helox chamber at low temperature
   80% He + 20% O_2 and T_a @ 0 °C

2. Animals become hypothermic after several hours
   @ 6-8 hours (T_{re} 7-10 °C)

3. Animals taken from helox chambers and placed in cold room,
   T_a 7 °C

4. Animals used for experimentation
   T_{re} 7 °C

5. Animals are rewarmed by removal from cold room (T_a 7 °C)
   to room temperature (T_a 22 °C)
   Normothermia @ 2 hours (T_{re} 37-38 °C)

Recently, under the guidance of Dr. Wynn Volkert, a unique
modification of the helium-cold method has been achieved. This
method incorporates the use of Halothane, an anesthetic fluoro-
carbon, in the initial steps, and an outline is presented in Figure 2.
The notable advantage to the helium + Halothane method is the
shortened induction time, about 2 hours or less.

FIGURE 2

STEPS IN HELIUM + HALOTHANE HYPOTHERMIA

1. ANIMALS IN RESPIRATORY CHAMBER AT ROOM TEMPERATURE (T_A @ 22°C) WITH 5% HALOTHANE + OXYGEN, @ 5 MIN. TO ANESTHESIA

2. ANIMALS IN COLD ROOM (T_A 0°C) @ 5 MIN. WITH 2½% HALOTHANE AND HELOX (80% HE + 20% O_2) THEN 25 MIN. (CLOSED SYSTEM) TO MILD HYPOTHERMIA (T_{RE} 22-23°C)

3. ANIMALS (T_{RE} 22-23°C) TRANSFERRED TO HELOX CHAMBER AT LOW TEMPERATURE, 80% HE + 20% O_2 AND T_A 0°C

4. ANIMALS BECOME HYPOTHERMIC AFTER @ 1 HOUR (T_{RE} 7-10°C)

5. ANIMALS TAKEN FROM HELOX CHAMBERS AND PLACED IN COLD ROOM, T_A 7°C

6. ANIMALS USED FOR EXPERIMENTATION, T_{RE} 7°C

7. ANIMALS REWARMED BY REMOVAL FROM COLD ROOM (T_A 7°C) TO ROOM TEMPERATURE (T_A 22°C), NORMOTHERMIA @ 2 HOURS (T_{RE} 37-38°C)

It is not unreasonable to mention that knowledge of the side effects of halothane is limited. We have assumed that most or all of the gas will be "blown off" during the prolonged period of hypothermia. This assumption remains to be tested. Plans are underway to test for the presence of Halothane in expired gases after various periods of time in hypothermia.

In the present experiments, i.e. a test for improved induction, some animals were also exposed to prior heat acclimation (T_a 34°C for two weeks) and then helium + Halothane induction was used.

Glucose and glycogen: Plasma glucose levels were determined by drawing ventricular samples and testing with a glucose oxidase method (Glucostat Preparation, Worthington, N.J.). Liver glycogen was estimated using an anthrone method described in detail by Carroll, Longley and Roe (1956).

RESULTS AND DISCUSSION

Induction times and survival: It has been found repeatedly that with a shortened induction time there follows an increased survival time. There is an inverse relationship between induction time and survival time. A summary of data is presented in Figure 3.

FIGURE 3

INDUCTION TIMES AND SURVIVAL
IN THE HYPOTHERMIC HAMSTER
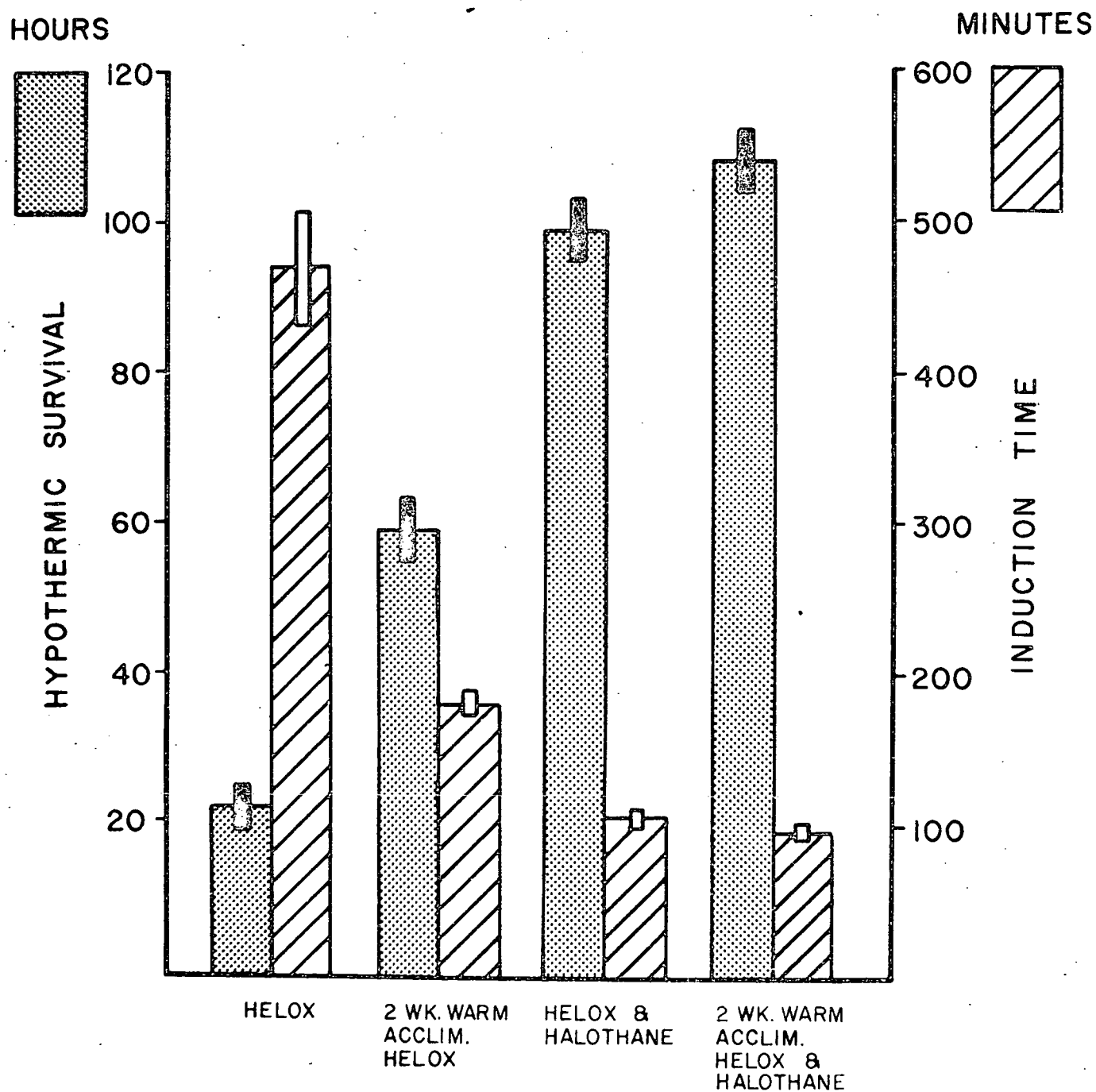
The data in Table 1 are a composite of results from the present study and two other investigations from this laboratory (Prewitt et al., loc. cit. and Musacchia, loc. cit.). It is of special interest to us that the use of halothane markedly reduces induction time. Furthermore, it is evident that prior heat acclimation does not significantly enhance the halothane assisted induction, viz. 96 $\pm$ 3 and 102 $\pm$ 4 minutes.

TABLE 1

HAMSTER Mesocricetus auratus			
Procedure		Induction Time (min)	Hypothermic Survival (hr) (T_{re} 7 °C)
Pre-Induction (T_a)	Induction		
21-24 °C	80:20 Helox * (11)	468 ± 40	22 ± 3
21-24 °C	80:20 Helox & Halothane (15)	102 ± 4	99 ± 4
2 wk 34-35 °C	80:20 Helox ** (34)	180 ± 3	59 ± 4
2 wk 34-35 °C	80:20 Helox & Halothane (15)	96 ± 3	108 ± 4

Previously reported * 1972, PSEBM, 140: 1279
** 1972, AJP, 222: 495

Number of animals ( ); X and SEM

Carbohydrate metabolism: Our initial studies of a metabolic limitation of survival suggested that a severe hypoglycemia, particularly at the terminal stages of hypothermia, could well be related to an incapacitation of the respiratory center (Prewitt et al. loc. cit.). An experiment was designed to "follow" changes in plasma glucose and liver glycogen at various stages of hypothermia. Figure 4 shows the main features of this experiment. The plasma glucose levels and liver glycogen values at various stages of hypothermia are summarized in Table 2.
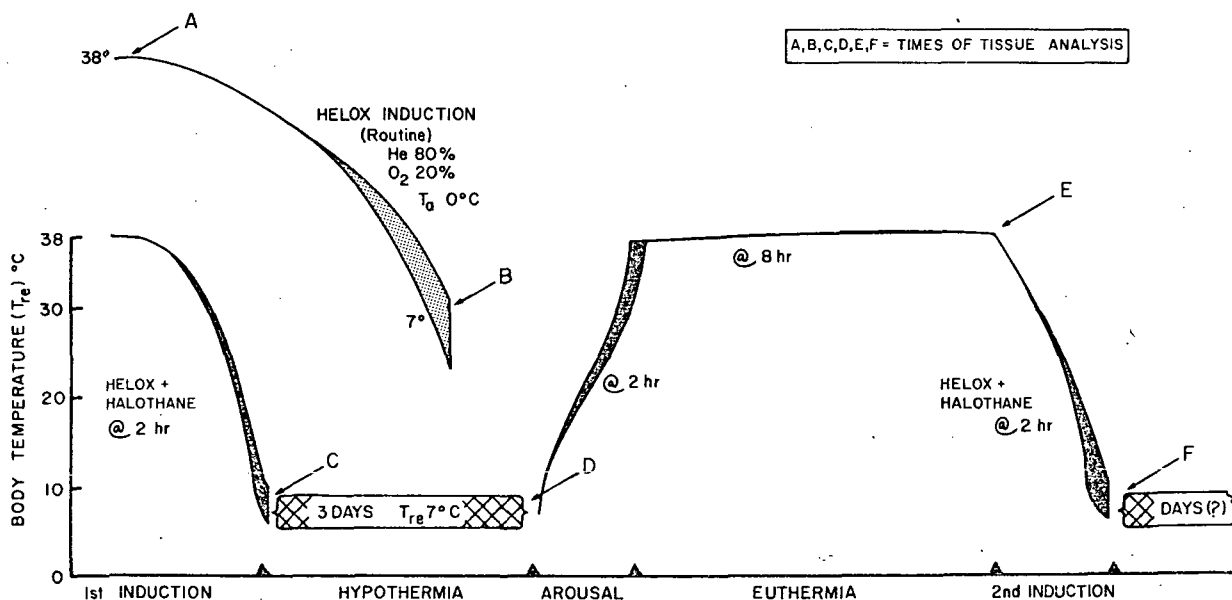
FIGURE 4

REPEATED INDUCTION OF HYPOTHERMIA IN HAMSTER *M. auratus*

TABLE 2

HAMSTER, Mesocricetus auratus, HYPOTHERMIA AND TISSUE ANALYSIS		
EXPERIMENTAL CONDITIONS	LIVER GLYCOGEN MG/G WET WT	PLASMA GLUCOSE MG %
A. NORMOTHERMIC (12)	69.2 ± 9.1	98 ± 8
B. HYPOTHERMIC (15) 80:20 HELOX	2.6 ± 1.8	31.3 ± 6.8
C. HYPOTHERMIC (5) 80:20 HELOX AND HALOTHANE	36.2 ± 2.1	286 ± 22
D. HYPOTHERMIC, 3 DAYS (5) 80:20 HELOX AND HALOTHANE	16.5 ± 3.4	78 ± 30
E. HYPOTHERMIC, 3 DAYS (5) 80:20 HELOX AND HALOTHANE (10 HR AWAKE)	1.18 ± .45	75 ± 9
F. HYPOTHERMIC, 3 DAYS (5) 80:20 HELOX AND HALOTHANE (10 HR AWAKE, THEN HYPOTHERMIC AGAIN)	0.61 ± 0.2	130.1 ± 24.9

There are several features of these data which warrant comments. It is clear that at all stages of hypothermia examined, there is a reduction in liver glycogen. Although there is a reduction in liver glycogen from control values of 69.2 ± 9.1 to 36.2 ± 2.1 mgs/gm wet weight in the helium + Halothane inductions, this is not as marked as the other values seen in other forms of induction. It

appears then that there is a sparing action in glycogenolysis when Halothane is used. In fact, it seems to persist even after 3 days in hypothermia. Plasma glucose levels also appear to be affected by induction hypothermia. However, one of the puzzling results of these studies is the fact that when Halothane is used to assist in induction of hypothermia, there is an elevation in plasma glucose values, e.g. experiments C and F (Table 2). It is of note that even after 3 days of hypothermia or after being awakened for 10 hours, the plasma glucose levels are only moderately depleted. The rise from 75 $\pm$ 9 mgs% in 10 hour awake animals to 130.1 $\pm$ 24.9 mgs% with a second induction is particularly interesting. Liver glycogen is greatly depleted in animals awakened from hypothermia. What then is the source of plasma glucose? Second inductions have been accomplished and hypothermic survival lasts for almost a day.

In these experiments we consider that we are in a highly innovative stage and interpretations would be highly speculative. We have elected to report the results precisely as they have been recorded and to point out that additional experiments are underway.

Blood glucose and survival: Another approach to the problem of the role of blood glucose and survival has been undertaken. Mr. Garth Resch, a doctoral student, is working on this project. Mr. Resch is a Ph.D. student in the Department of Physiology. He has devoted a considerable effort to the development of these procedures. His research has been supported not only by this NASA

grant, but also with funds from an NIH Environmental Physiology Training Grant.

The experimental hypothesis developed at this time is: If glucose is essential to sustain hypothermia, then replenishment during hypothermia should improve survival.

Several experimental approaches were used, and two showed promise. In one approach glucose was administered by direct injection into the carotid via a PE 10 cannula. Glucose, 225 mg/ml in physiologic saline, was given 0.1 ml at 4 hour intervals. In brief, the hamsters, about 100 to 130 gm, received about 0.5 to 0.6 ml of saline and a total input of about 13.5 mgs of glucose.

A second approach was to use a constant infusion pump which could be geared-down to an extremely slow rate. Again the carotid was cannulated with PE 10 and glucose 225 mg/ml was infused at a rate of 0.012 ml/hr. This apparatus was improved to the point where six infusions could be done simultaneously. In addition, controls, i.e., infusions of saline without glucose added, could be run simultaneously with test subjects. An illustration of this experimental set-up is provided in Figure 5. It must be added that surgical procedures for cannulation were done while the animal is in hypothermia at T_{re} 7°C.

The results of glucose administration clearly showed that survival was enhanced significantly. The data from our first experiments are summarized in Figure 6.

FIGURE 5

Hamster: M.auratus T_{re} 7°C T_a 7°C
Carotid infusion, PE 10 cannula
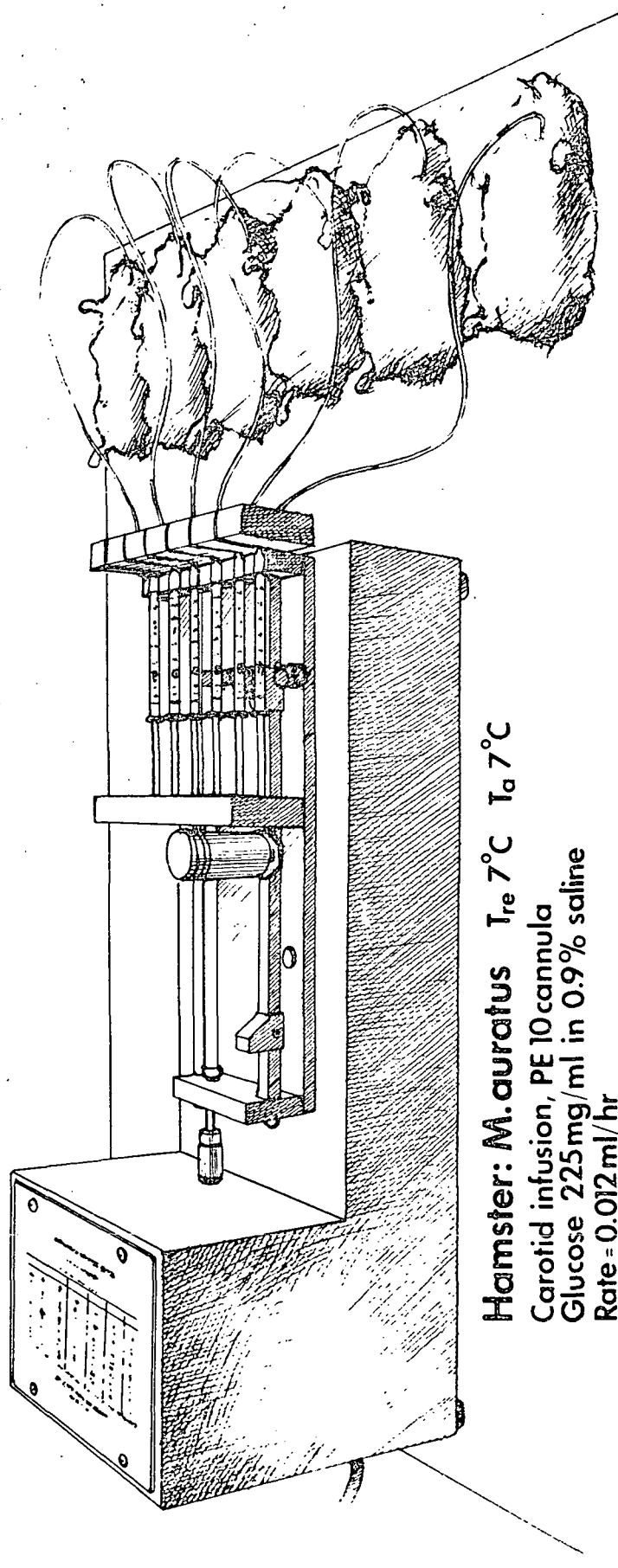Glucose 225mg/ml in 0.9% saline
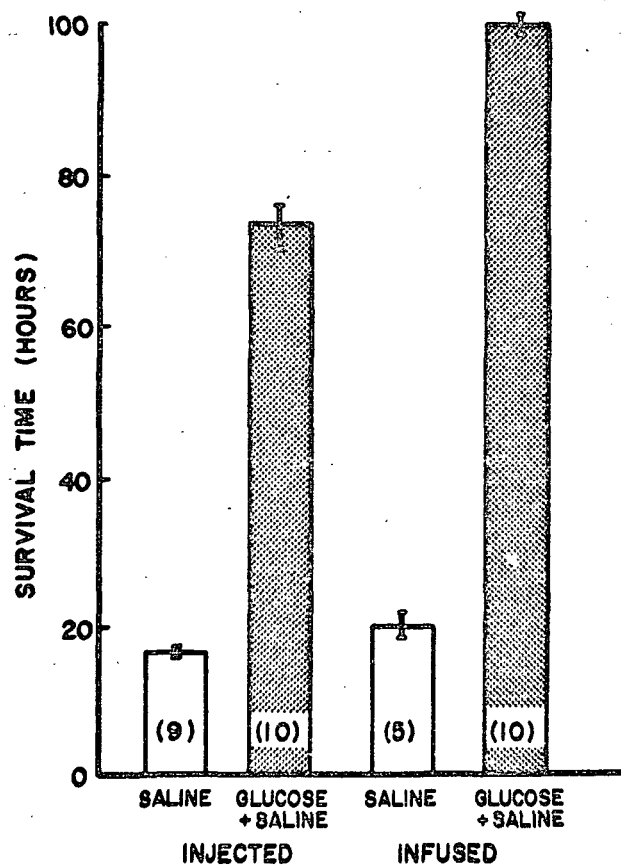Rate = 0.012ml/hr

FIGURE 6

SURVIVAL TIME IN HYPOTHERMIC HAMSTERS

GLUCOSE (225 mg/ml) ADMINISTERED BY INJECTION (4 hr PERIODS) OR BY CONSTANT INFUSION

We believe the results of these experiments may provide substantive data that glucose is a key factor in hypothermic survival. On the premise that glucose is essential for adequate function of the central nervous system and since respiratory arrest (Anderson et al. 1971 and Prewitt et al., loc. cit.) appears to be intimately involved with hypothermic demise, it is a logical deduction that the role of

glucose appears to be focused on maintenance of the CNS respiratory center. Despite the fact that several years ago Popovic (1955, 1959) reported that administration of glucose to the rat in hypothermia was ineffective in prolonging survival, we propose that administration of glucose and perhaps other metabolites may provide experimental approaches for studies of prolonged depressed metabolic states in the hamster.

CONCLUSIONS

1. A combination of helium and halothane can be used to induce hypothermia in the hamster.
2. Under conditions described in this research, the helium-Halothane method reduces the time of induction to about 2 hours or less.
3. The time required for induction is inversely related to survival time.
4. Glucose supplied directly to the circulatory system by infusion is relatable to improved survival time. Blood glucose, an essential factor in CNS metabolism, appears to be important in hypothermic survival.

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HELIUM-COLD INDUCED HYPOTHERMIA IN THE WHITE RAT.

X. J. Musacchia and Mollie Jacobs

INTRODUCTION

One of the best known characteristics of helium, as a diluent atmospheric gas, is its high thermoconductivity (six to seven times that of nitrogen). When it is used to replace atmospheric nitrogen, in mammals, a variety of physiologic adjustments take place. One of these adjustments is an increased level of metabolism, as seen in measurements of oxygen consumption and/or increased rates of respiration. These features have been reported in several different laboratory animals: rabbits (1), rats (2,3), mice (4), and hamsters (5).

In a series of investigations using the hamster, Mesocricetus auratus, we have utilized the high thermoconductivity feature and the resultant increase in metabolic expenditure in challenging the thermoregulatory capacity of the hamster (5,9). In these experiments, animals were exposed to low ambient temperatures of about 0°, and a gaseous atmosphere of 80% helium and 20% oxygen (helox). For convenience, this procedure is herein termed the "helium-cold method" for induction of hypothermia. After a period of a few hours, hamsters

lose their ability to thermoregulate and they undergo hypothermia. The fall in body temperature, to about 7° for hours at a time, is evidently not unusually insulting since upon exposure to room temperatures, about 22°, these animals readily return to normal body temperatures, circa 37-38°.

It is essential to point out a major difference between the hamster, the preferred animal in earlier experiments, and the white rat. The hamster is generally considered to be a facultative hibernator whereas the rat is a non-hibernator. Since the white rat has often been used for studies of hypothermia (11,12,13), we decided to expand our investigations of the helium-cold method for induction of hypothermia through exploring its use on the rat.

In this paper, the results of several experimental approaches are described and two types of survival are examined: "biological survival" in which revival from hypothermia to normothermia is achieved, and "clinical survival" in which one or more functional attributes (e.g, heartbeat, respiration or sensory reflexes) are monitored in the hypothermic animal until it dies. Both terms have been described in greater detail by Popovic (14). Our principal objectives were to examine hypothermic survival utilizing the helium-cold method for induction of hypothermia in the Sprague-Dawley white rat and, secondarily, to make comparisons between helium-cold hypothermia and other forms of hypothermia in this animal. An additional series of comparisons were made with results of previous reports using the golden hamster.

MATERIALS AND METHODS

The rats used, <u>Rattus norvegicus</u>, (Sprague-Dawley), were males obtained commercially (Carworth, Inc.). They were allowed to adjust to our animal room facilities for several days prior to being used in an experiment. Subsequently, individual animals were placed in hypothermic induction chambers at ambient temperatures of 0°. In these chambers there was a continuous gas flow of helox at a rate of about 150 ml per minute. The method has been described in detail elsewhere (5,9). Upon reaching lowered body temperatures, the rats were then removed to a cold room or a refrigerator with ambient temperatures maintained at either 14 or 16°.

In experiments dealing with biological survival animals were maintained in a cold room at T_a 14° and then, after 4, 6, or 8 hrs in hypothermia, removed to room temperatures of 21 and 22°. In two experimental groups a heated air flow was used to aid rewarming A Hoover hand hair dryer was used to blow warm air directly at the animal (temperature at the surface of the animal was 50 to 70°) from approximately 6 to 8 inches away.

Body temperatures of animals being rewarmed at room temperature were recorded with an Esterline-Angus recorder and copper constantan deep rectal probes. Survival time, i.e. clinical survival, was monitored by using a Lead II ecg and recording heart beats. A Grass 7 polygraph was used.

In experiments concerned with spontaneous rewarming and subsequent survival of animals maintained at an ambient temperature of 16°, rectal temperatures were taken at intervals of 15 to 30 min using Schulties thermometers or telethermometer rectal probes (YSI).

RESULTS

Survival time in hypothermia. In this series of experiments rats were maintained in hypothermia at T_{re} circa 14-15° and at ambient temperatures, T_a 14°, until they died. Twelve animals, 117 to 177 grams, which were induced into hypothermia (143.8 ± 4.44 min), lived for periods ranging from 8.5 hrs to over 12 hrs; their mean survival time was 10.12 ± 0.36 SEM hrs.

Survival following hypothermia. In order to investigate the ability of rats to survive after rewarming from various lengths of time in hypothermia at T_{re} circa 14-15°, four series of experiments (A,B,C and D) were performed. The results are summarized in Table 1.

Series A contained 18 rats made hypothermic for four hrs after which they were removed to room temperature T_a 21-22° in order to reestablish normothermia. Sixteen of the eighteen survived and appeared normal several hours later. The two that did not survive rewarming had varied from the remainder of the group in that they had a somewhat lower body temperature upon removal from the helox. In both animals body temperatures had fallen to T_{re} 12.5°, whereas the lowest induction temperatures T_{re} for those which survived was 13°.

Table 1. Hypothermia maintained at cold room temperature; T_a 14°. Comparisons of the effects of total time in hypothermia and rewarming technique on the percent survival after rewarming. Values given are mean ± SEM; number of animals per group in ( ).

Series	Weight (g)	Induction Time (min)	Induction T_{re} (°C)	Time in Hypothermia (hr)	Rewarmed with T_a (°C)	% Survival
A (18)	132.6 ± 4.01	147.8 ± 4.92	14.1 ± 0.22	4	21-22	88%
B (19)	136.3 ± 2.61	147.6 ± 3.53	14.2 ± 0.23	6	21-22	5%
C (20)	136.0 ± 3.74	141.2 ± 3.01	14.5 ± 0.28	6	50-70	25%
D (15)	141.6 ± 4.20	141.0 ± 4.56	14.5 ± 0.29	8	50-70	0%

Series B consisted of 19 animals, of which only one survived the rewarming. Animals in this group were hypothermic for six hrs before rewarming was started. In general, after two to three hrs, body temperatures increased to <u>circa</u> T_{re} 22°. This applied to the one that survived as well as those that died. In most cases death occurred between the second and third hr after the animal was removed from the cold.

Rewarming to normothermia varied considerably. However, it was evident that the animals in Series A rewarmed faster than those in Series B. Rectal temperatures were recorded in 12 animals from each group until they reached equilibrium with room temperature, i.e. about 22°. The rats which had been maintained in hypothermia for only four hrs rewarmed to T_{re} 22-23° in less than two hrs, whereas those which had been hypothermic for six hrs required about two to three hrs to reach T_{re} 22-23°.

Series C consisted of 20 animals which were hypothermic for six hrs and rewarmed with the aid of a flow of heated air. Within five min after this rewarming began, animals showed a gasping reflex and a gradual increase in respiration rate. Often a rat would move its front paws and head to some extent. Those that survived rewarming turned themselves upright within 30 min, at which time their body temperatures ranged from 30° to 37.5° T_{re}. Of the 20, five survived rewarming; however, in most of the animals the tail and front and hind limbs became darkened and swollen,

indicating tissue anoxia. In some rats there followed a necrosis and sloughing of tissue. One animal, which had been positioned with its dorsal surface directly in the path of the heated air flow, showed the tissue degeneration on the tail only. In this animal, both front and hind limbs were normal.

Series D contained 15 rats which were rewarmed, also with the aid of the external heated air flow, after eight hrs in hypothermia. None of the 15 survived longer than one hr, and 10 of the 15 died within 30 min. Animals in this group showed much less activity during the rewarming period, although they did show movements similar to those seen in other rats. None of these animals righted themselves before death. Four in this group failed to respond to external heat and might possibly have died at the time the rewarming began. These were four of five rats in which body temperatures were lowered to between T_{re} 13.0 and 13.5° during induction. The remaining animals in this group were removed from helox at T_{re} 14.3 to 16.2°. Because a number of animals in this series lacked eye reflexes and some had an opaque whitening of the cornea and iris, several were tested with a Lead II ecg prior to rewarming; they showed a definite, although slight, heartbeat.

Hypothermia, T_a 16°, and spontaneous arousal. Sixty rats, 109 to 217 grams, were induced into hypothermia with body temperature reaching, T_{re} 13.5 to 17.0°. Upon removal from helox to a maintaining cold room, T_a 16°, these animals' body temperatures began to

rise within several minutes. Sixty-five percent survived after reaching normal body temperature, T_{re} 37°. Figure 1 below shows typical temperature curves of five rats rewarming under these conditions.

FIGURE 1
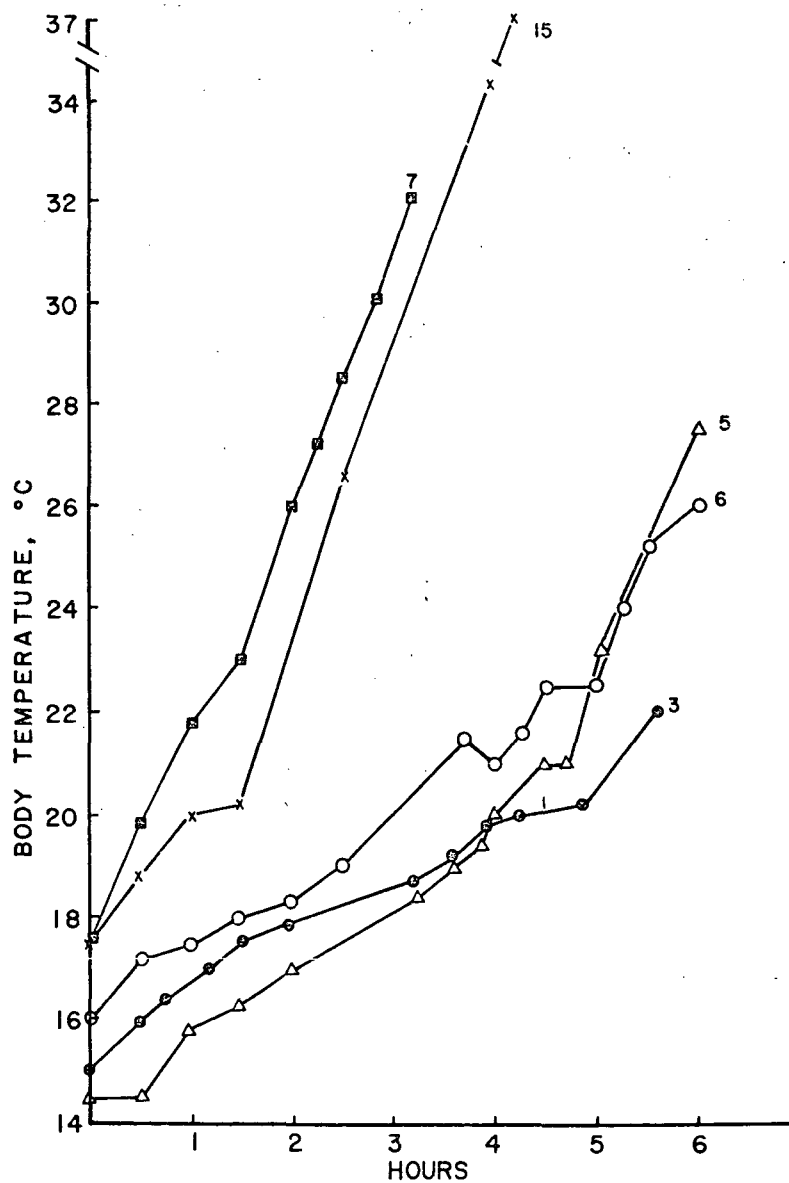
TEMPERATURE RISE IN SPONTANEOUSLY REWARMING HYPOTHERMIC RATS
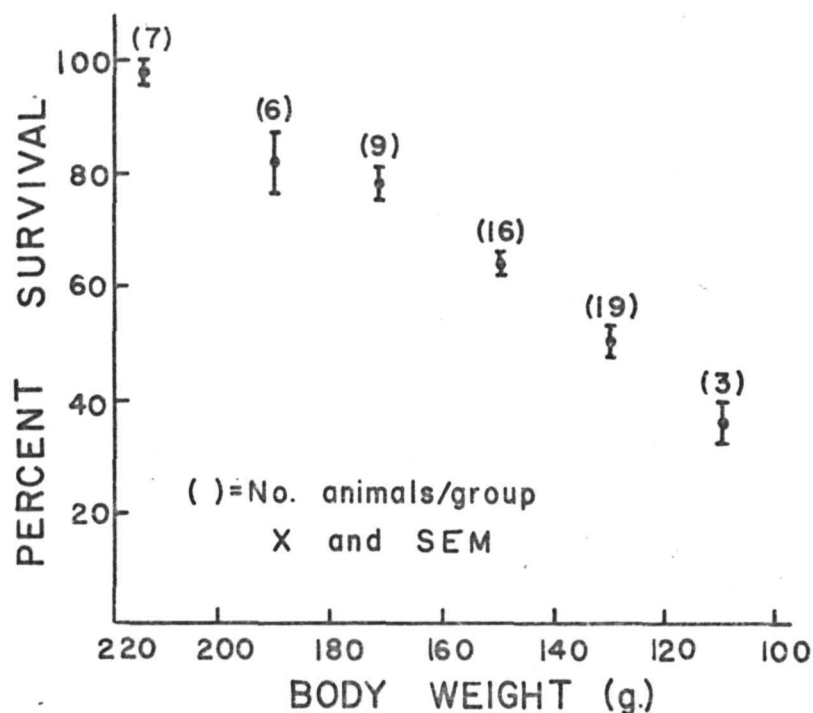(T_a 16° C)

When these 60 rats are divided into weight groups with each group having a range of 20 grams, two interesting trends become evident. As shown in Table 2, there is a direct relationship between body weight and the increasing amount of time in helox necessary for an animal to reach the desired low body temperature. This has also been observed in hamsters (Musacchia, unpublished). The second trend is that with an increase in animal weight, there is also an increase in percent survival. The relationship is linear as seen in (Figure 2).

Table 2. Temporary hypothermia at cold room temperature, T_a 16°. Rats are grouped according to body weights. Values given are mean ± SEM, number of animals per group in ( ).

Group	Weight (g)	Induction Time (min)	Induction T_{re} (°C)
100-120 (3)	111 ± 2	115 ± 8	15.7 ± 0.2
120-140 (19)	130 ± 1	126 ± 3	15.4 ± 0.2
140-160 (16)	148 ± 2	144 ± 5	15.5 ± 0.3
160-180 (9)	170 ± 2	187 ± 8	16.0 ± 0.3
180-200 (6)	189 ± 3	211 ± 13	14.9 ± 0.5
200-220 (7)	212 ± 1	240 ± 12	15.2 ± 0.6

FIGURE 2

Relationship between body weight and percent survival
of rats from hypothermia (T_{re} 13.5-17.0 C) after
spontaneous rewarming at T_a 16° C.

DISCUSSION

The experimental results showed that the laboratory white rat
can be inducted into hypothermic depressed metabolism using the
helium-cold method. In many ways this method appears to produce
a hypothermic state in the rat quite similar to that resulting from

such techniques as ice water immersion or hypercapnia + hypoxia.
For instance, it was also reported by Andjus and Smith (15) that
rats cooled to a low body temperature (15° or lower) will begin to
rewarm spontaneously if maintained in the cold at T_a 16° or above.
On the other hand, an animal removed from helox with a T_{re} as high
as 17.5°, will continue into hypothermic depression (T_{re} circa 15°)
as long as ambient temperature is 14°. Spontaneous rewarming
appears to be a consequence of the temperature of T_a in the cold
room rather than body temperature during induction or body temper-
ature upon removal from helox. Body temperature during induction
does, however, appear to have an effect on the rate of spontaneous
rewarming (Figure 1) and, if T_{re} falls to 13.5° or below, biological
survival is affected. This is evidenced by the four animals in
Series D in which the body temperatures had fallen to 13-13.5°
during induction. These animals did not respond to external
heating; they did not revive. In addition, the two animals in
Series A with T_{re} less than 13° were the only ones that did not
survive rewarming after four hrs in hypothermia.

In this report the efficacy of the helium-cold method was
characterized by clinical and biological survival. The first was
measured using survival in the hypothermic state as the parameter,
while in the second, revival from hypothermia and return to the
normothermic state was the critical factor. In comparison with re-
sults obtained in 1959 by Popovic (12), our data suggests a slight

enhancement in clinical survival at T_{re} 14-15°, viz. 8.5 to 12 hrs
with a mean of 10.1 hr. He found that at 15° body temperature,
rats survived 9.5 hrs.

Biological survival of helium-cold hypothermic rats is prob-
ably very similar to that reported by Popovic (14). He reported
that 50% of the animals recovered upon rewarming after 5.5 hrs at
T_{re} 15°, while we have shown that 88% survive after four hrs and
25% after six hrs of hypothermia. The latter were aided in rewarm-
ing with a heated air flow. Thus, we estimate that 50% would
survive somewhere in between four and six hrs. It is reasonable
to say that, in light of the present evidence, helium-cold has only
modest advantages for the induction of hypothermia as compared
with other methods of induction in the rat, e.g. icewater immersion
or hypercapnia + hypoxia.

Rewarming techniques do, however, appear to have an important
effect on biological survival. In these experiments we elected to
use only the most elemental processes to induce rewarming, viz. ex-
posure to room temperature (21-22°) and exposure to warmed (50-70°)
room air. In effect, these are "whole body" warming procedures. As
was shown, a larger percent of experimental animals survived six hrs
at a low T_{re} if they were aided in rewarming with more intense
outside heat. However, since most of those that did survive showed
ill effects, such as necrosis in areas of the feet or portions of
the tail, a better rewarming technique is required. Andjus and

Smith (15) have shown the necessity for differential warming in order to insure survival in the rat: in short, the need for initial localized cardiac rewarming followed by heat applied to the whole body. They also showed that there may well be the need to support the respiratory system when the rat is in a severe depressed metabolic state.

The survival and body weight relationship shown in Figure 2 is of particular interest. The heavier animals were adults and the smaller animals were juveniles or young adults. It has been known for some time that young, newborn rats can withstand and readily recover from hypothermic states (16). In very young animals, temperature regulatory mechanisms are not yet developed. In all of the rats used herein, temperature regulation is well established. Reasons for the prominent differences between the older, heavier animals and the younger, lighter animals are not known. It is not unreasonable, however, to speculate that the older animals have a greater thermogenic capacity, in part relatable to a greater abundance of brown fat. In hamsters, hypothermic survival and perhaps recovery is relatable to available carbohydrate (10) and modified thermoregulatory components (9). By way of comparison, brown fat tissue is known to have a predominant role in thermogenesis when the rat is exposed to cold (17), whereas the role of brown fat in the hamster is less well understood.

Clearly, the rat and hamster differ dramatically in their response to helium-cold hypothermia, as they may well differ in terms of other forms of hypothermic induction. For example, in comparison to the hamster, the rat is far less able to sustain prolonged hypothermia, since the hamster readily survives T_{re} 7° for one or two days (9,10). In our opinion, the helium-cold method for induction of hypothermia in the rat offers a potentially useful experimental tool for depressed metabolism studies.

SUMMARY

Exposure to a helox mixture of 80% helium and 20% oxygen and low ambient temperature induces hypothermia in Sprague-Dawley white rats. Hypothermia to body temperatures of about T_{re} 14-15° in the white rat is tolerated for various periods, for example, after 4 hours 88% survived and after 6 hours 25% survived. Rewarming to normotheric body temperature is achieved by exposure to room temperature, _circa_ 21-22°. Additional sources of external heat only moderately enhanced survival after rewarming. There is a direct relationship between body weight and percent survival. Despite the fact that they require a longer period in order to become hypothermic, the heavier animals are better able to survive. Spontaneous rewarming from low body temperatures of T_{re} 13.5 to 17° to normothermic body temperatures, T_{re} 37°, occurred routinely in

those animals placed at 16° ambient temperatures. Survival rates
were substantial, 65% of the 60 animals used in the latter experi-
ment survived indefinitely. It was concluded, within limits, that
spontaneous rewarming is a consequence of the ambient cold room
temperature rather than the body temperature reached during induc-
tion. Comparisons with previously published data concerned with
other methods of induction of hypothermia in the white rat indicate
that the helium-cold method herein described is slightly more effi-
cacious. The white rat is in no manner comparable to the hamster,
viz., in terms of depth of body temperature, T_{re} 7° and lengths of
one to three days of hypothermia induced by this same method.

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RADIATION RESPONSE AND INTESTINAL ABSORPTION IN THE GERBIL, MERIONES
UNGUICULATUS.

X. J. Musacchia and Marilyn Spate

INTRODUCTION

This is a continuation of the report presented in my Semi-Annual
Status Report, January 14, 1972. An extensive review of the liter-
ature and a statement of objectives were presented in that report.

MATERIALS AND METHODS

The Mongolian gerbils, Meriones unguiculatus, used for these
experiments are taken from a closed colony which we established
several years ago in the Space Sciences Research Center.

Glucose analysis of intestinal lumenal contents and whole
blood were measured by an enzymatic glucose oxidase method (Gluco-
stat from Worthington Biochemical Corporation, Freehold, N.J.) and
hematocrits were done with micro-capillary methods. The irradia-
tion source is a ^{60}Co gamma source; the instrument used throughout
our NASA sponsored projects.

Whole body dose survival data are obtained concomitantly with intestinal absorption analyses. The progress of the entire experiment is directly related to breeding and colony size. Thus, experiments are done as animals become available.

RESULTS

Whole body irradiation responses. With irradiation exposures at 1100 Rads there was 100% survival at 30 days. The LD 50/30 appears to be about 1250 Rads (Figure 1).

Intestinal absorption in vivo. Glucose absorption is relatively unaffected one and two days after whole body exposures to 1250 Rads. In contrast, on days 3, 7 and 14 post-irradiation there is an increase in glucose absorption (Figure 2).

In the last report, the data for 3 days post-irradiation suggested that there was no significant difference from the values obtained in control, non-irradiated subjects. The increased number of animals in the current project obviously resulted in a difference which appears to be a post-irradiation response.

Blood. Blood glucose values suggest two responses following whole body exposures to 1250 Rads, viz. an initial rise during days 1, 2 and 3 and a fall on days 7 and 14. The hematocrit values show, for the most part, an inverse relationship to the blood glucose values (Figure 3).
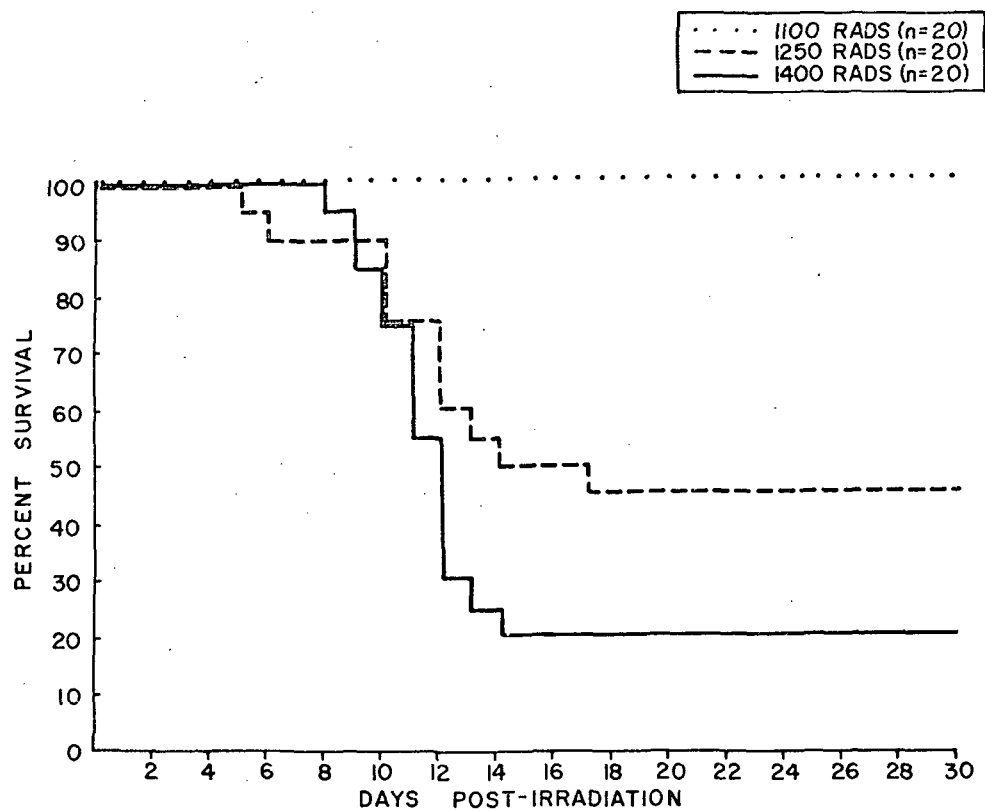
FIGURE 1

PERCENT SURVIVAL OF GERBILS AFTER ^{60}Co WHOLE BODY IRRADIATION

FIGURE 2

ABSORPTION OF D-GLUCOSE IN VIVO IN GERBILS
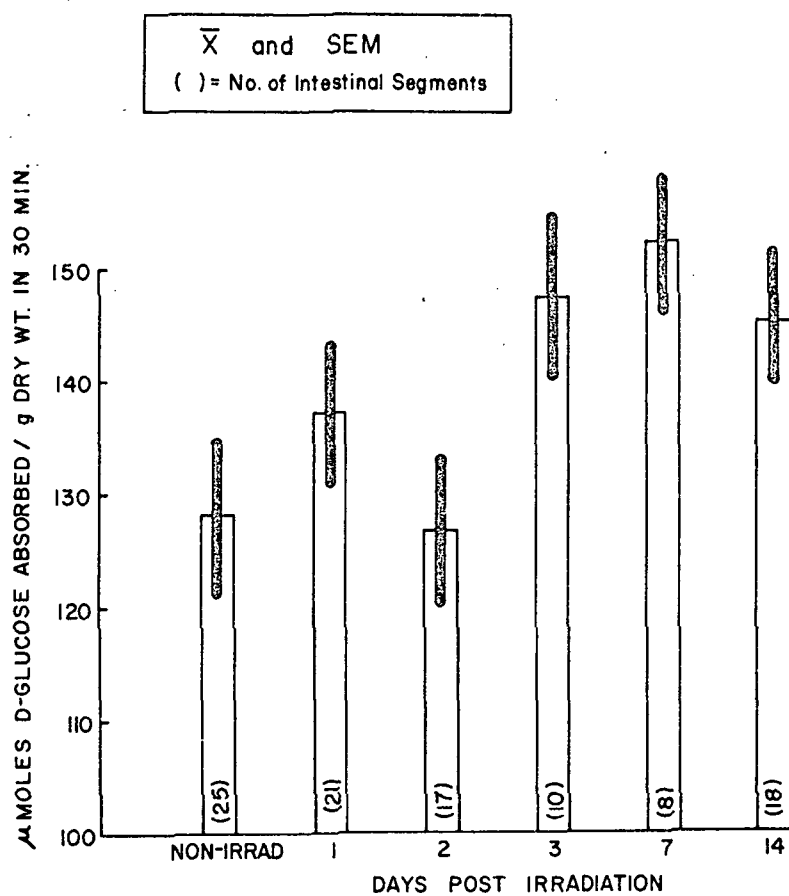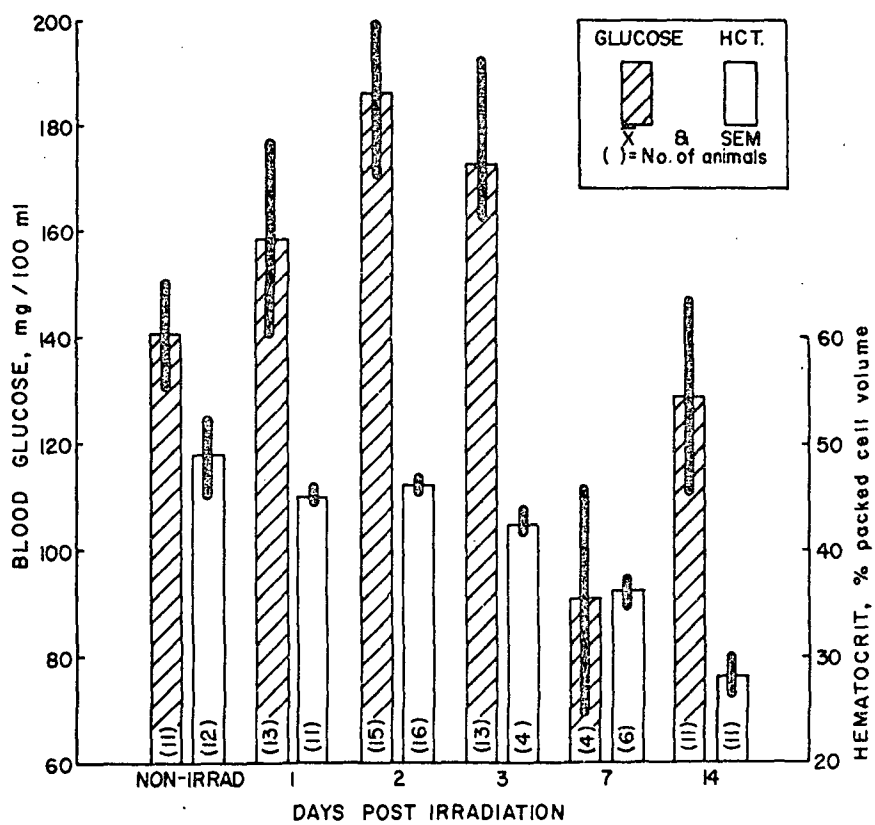AFTER IRRADIATION, 1250 RADS (whole body exposure, ^{60}Co)

FIGURE 3

BLOOD GLUCOSE and HEMATOCRIT in GERBILS
after IRRADIATION, 1250 RADS (whole body exposure, ^{60}Co)

DISCUSSION AND SUMMARY

In the present investigation of dose response curves, we performed an irradiation experiment at 1100 Rads. The data in Figure 1 clearly shows that at this dose there is 100% survival. This result supported our earlier contention that whole body exposures at 1250 Rads, i.e. where the LD 50/30 levels were evident, should be more than adequate to test an absorption response within the gastrointestinal radiation syndrome.

The post-irradiation gastro-intestinal response, in terms of capacity for glucose absorption, indicates that the gerbil more closely approximates the hamster and ground squirrel rather than the rat. A detailed review of this subject was presented in the January 1972 report and would be redundant at this time.

Because of the uniqueness of the finding that glucose absorption is increased in post-irradiated gerbils one more series of experiments are planned. During the next several months a series of in vitro tests of the gut from irradiated animals will be made. If the results tend to confirm or correlate our present findings, the data will be prepared as a manuscript for publication. Hopefully, this will serve to provide a new outlook on post-irradiation intestinal responses. The "new" outlook would be, in effect, a focus on the need for caution in accepting textbook generalizations about post-irradiation depressions of intestinal absorption.

A literature review of radiation responses in the gerbil was presented in my Semi-Annual Status Report, January 14, 1972. Repetition is unwarrented in the present report.

SECTION II . . . . CONTENTS

A. Journal Articles (Published and in press)

1. Role of Heat and Cold Acclimation in Helium-cold Hypothermia in the Hamster, Mesocricetus auratus. X.J. Musacchia, Am. J. Physiol. 222:495-498, 1972.

2. Seasonal changes in the Fatty Acid Spectrum in Hibernating and Non-hibernating Ground Squirrels, Citellus tridecemlineatus. W. S. Platner, B. C. Patnayak and X. J. Musacchia. Comp. Biochem. Physiol. 42:927-935, 1972.

3. Evidence for a Metabolic Limitation of Survival in Hypothermic Hamsters. Russell L. Prewitt, Gary Anderson and X. J. Musacchia Proc. Soc. Exper. Biol. Med. 140:1279-1283, 1972.

4. Post-Irradiation Hibernation and Radiation Response of Ground Squirrels: Telemetry Surveillance. R. E. Barr and X. J. Musacchia Rad. Res. 51:631-637, 1972.

5. Synergistic Role of Temperature and Exposure to onizing Radiation. X. J. Musacchia, W. A. Volkert, R. Prewitt, R. Chandler and M. Spate Proc. Internat. Symp. Envirn. Physiol., Bioenergetics, 179-184. (Eds. R. E. Smith et al.) FASEB, Washington D. C., 1972.

6. Hypoxia an Adjunct in Helium Cold Hypothermia: Sparing Effect on Cardiac Metabolites. G. L. Anderson, G. E. Resch and X. J. Musacchia Am. J. Physiol. (in press) 1972. (Copy of manuscript was submitted in January 14, 1972 Semi-annual States Report)

Heat and cold acclimation in
helium-cold hypothermia in the hamster

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MUSACCHIA, X. J. *Heat and cold acclimation in helium-cold hypothermia in the hamster.* Am. J. Physiol. 222(2): 495–498. 1972.—A study was made of the effects of acclimation of hamsters to high, 34–35 C, and low 4–5 C, temperatures for periods up to 6 weeks on the induction of hypothermia in hamsters. Hypothermia was achieved by exposing hamsters to a helox mixture of 80% helium and 20% oxygen at 0 C. Hypothermic induction to T_b 7 C was most rapid (2–3 hr) in heat-acclimated hamsters and slowest (6–12 hr) in cold-acclimated hamsters. The induction period was intermediate (5–8 hr) in room temperature nonacclimated animals (controls). Survival time in hypothermia was relatable to previous temperature acclimations. Heat-acclimated hamsters lived longest (2–2.5 days), cold-acclimated animals were short lived (12–18 hr), and nonacclimated control animals were intermediate (18–24 hr). The longevity of hypothermia in heat-acclimated hamsters compares favorably with the periods of hibernation experienced by this species. The hypothesis that thermogenesis in cold-acclimated hamsters would accentuate resistance to induction of hypothermia was substantiated.

hypothermia induction; hypothermic viability

THE USE OF EXPOSURE TO COMBINATIONS OF HELIUM and cold for the induction of hypothermia has been described for hamsters (5, 10) and albino mice (13). A variety of physiologic parameters have been described in the hamster: behavior, respiration, and body temperature responses (5) and blood gas changes (12); in the albino mouse: oxygen consumption, respiration rates, and body temperatures (13) were also described. The role of acclimation to high and low temperature prior to induction of hypothermia was investigated and an initial report was made (9); details are herein reported.

The continued use of the hamster in our experiments is based in great part on its ability to acclimate to both high and low temperatures. In recent experiments (11) we showed that hamsters readily tolerated a temperature of 5 C for periods up to 10 weeks. Adaptive changes were seen in terms of behavioral adjustment, intestinal absorption, and continued gain in body weight. The hamster's ability to acclimate to high temperatures, 35 ± 1 C for periods of several weeks has also been demonstrated (2, 3). There are adaptive changes in terms of reduced respiratory enzyme activities and lowered metabolic rates. These modifications were examined in hamsters which were then exposed to even higher temperatures and which showed increased heat tolerances. Thus, the hamster appears to be well qualified for studies of

physiologic adjustment and/or responses to temperature variations.

It was considered worthwhile to examine the hamsters ability to acclimate to a high or low environmental temperature and to relate its thermoregulatory responses to the induction of hypothermia using the helium-cold technique (5, 10). The experimental hypothesis was that a cold-acclimated animal would be able to resist induction of hypothermia much more readily than a heat-acclimated animal. The results of the following experiments bore out the hypothesis, and at the same time provided an unexpected result, namely, that survival in hypothermia is directly related to shortened induction time. In addition, the experimental approach herein described provides a method for rapid induction of hypothermia and a prospective minimization of biological insult.

MATERIALS AND METHODS

Hamsters, *Mesocricetus auratus*, from our closed colony were used. Whenever possible, siblings were used for the three experimental groupings: *a*) room temperature control subjects, at T_a 22–23 C; *b*) heat-acclimated subjects, at T_a 34–35 C; and *c*) cold-acclimated subjects at T_a 4–5 C. Both males and females 85–100 g were used, but maintained separately to avoid variations due to mating or gestation. Heat and cold exposures extended from 1 to 6 weeks. Hamsters from each group were used concomitantly. Helium-cold hypothermia consists of exposing an animal to a mixture of helium (80%) and oxygen (20%) at low ambient temperatures, ca. 0 C. The gas mixtures of helium and oxygen is herein referred to as helox.

The experiment was designed such that the animals were weighed immediately at the start of the heat or cold acclimation. Each animal was weighed again at the time it was placed into the helium-cold chambers and upon removal from the chambers. Only the latter weight data warranted reporting as essential to interpreting results.

The method for induction of hypothermia was changed slightly from the original description (5). The temperature of the helium-cold chambers was 0 C. The flow rate of helox was about 150 ml/min. Body temperature was measured in each animal at a time when the animal showed loss of postural control in the helium-cold chambers, generally, at T_b 10–12 C. At T_b 10 ± 1 C hamsters were removed from the helium-cold chambers and kept in a cold room at T_a 0 C until body temperatures reached 7–8 C. At that time, each
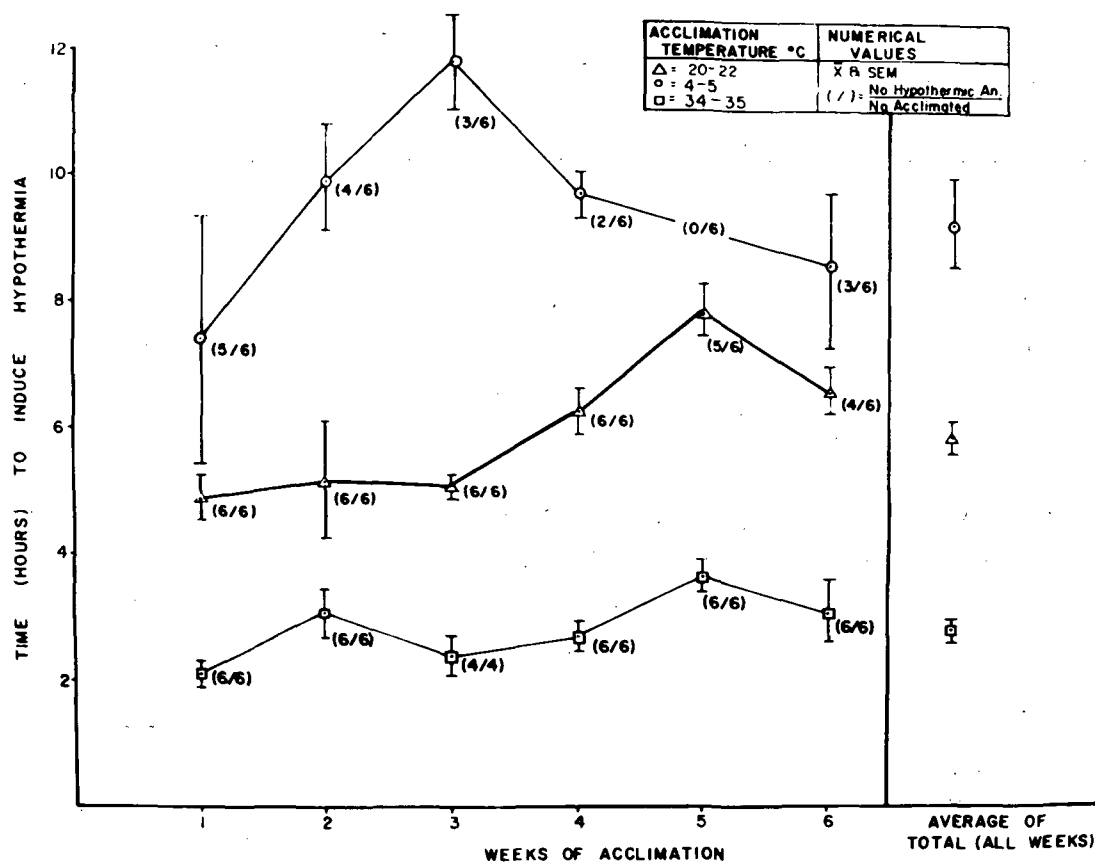
FIG. 1. Helium-cold hypothermia (T_b 7 C) induction time of heat- and cold-acclimated hamsters.

animal was removed to a household refrigerator at T_a 7 C. In these procedures an animal was in helox only during its stay in the helium-cold chamber. The cold room and the refrigerator are aerated with the normal mixtures of gases of the ambient atmosphere. The terminal period for helium-cold exposure for any given animal was set at 12 hr. If after that period an animal was not yet hypothermic, it was removed from the helium-cold chamber and no longer included in these experiments.

Deep rectal temperatures were monitored periodically with Wesco rapid-recording thermometers (Schultheis Co., N.Y.C.) or Yellow Springs Instrument telethermometer recording thermometers. Hamsters remained at low T_b, 7–8 C until death.

RESULTS

The time for induction of hypothermia using the helium-cold method is relatable to the experience of previous heat and cold acclimation in the hamster. Heat-acclimated animals became hypothermic in relatively short periods, 2–3 hr. Cold-acclimated animals required the longest periods of time to achieve hypothermia, and some did not become hypothermic within the 12-hr period of helium-cold exposure (Fig. 1). In those groups (Fig. 1) where the numerator and denominator are identical (e.g., 6/6) all the hamsters became hypothermic within 12 hr; otherwise, where the numerator is less than the denominator, a number of hamsters did not undergo hypothermia within that period. Hamsters maintained at room temperature required periods which ranged 5.5–7 hr to become hypothermic.
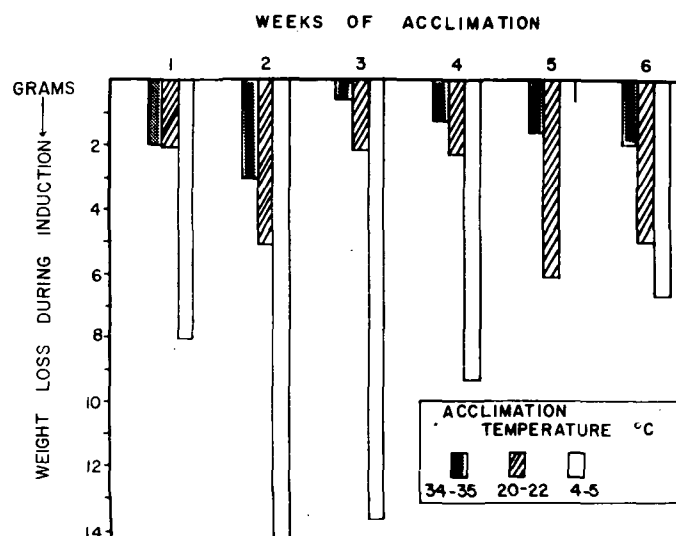

FIG. 2. Loss of weight during induction of helium-cold hypothermia in heat- and cold-acclimated hamsters.

With the induction of hypothermia there is a loss of body weight; data are presented (Fig. 2). Heat-acclimated hamsters show the smallest weight loss during induction of hypothermia, whereas the cold-acclimated animals show the greatest weight losses. Intermediate levels of weight loss occurred in the control animals.

The viability of hamsters while in a state of hypothermia has been a prime objective in these studies and the results of temperature acclimation are presented (Figs. 3 and 4). It is
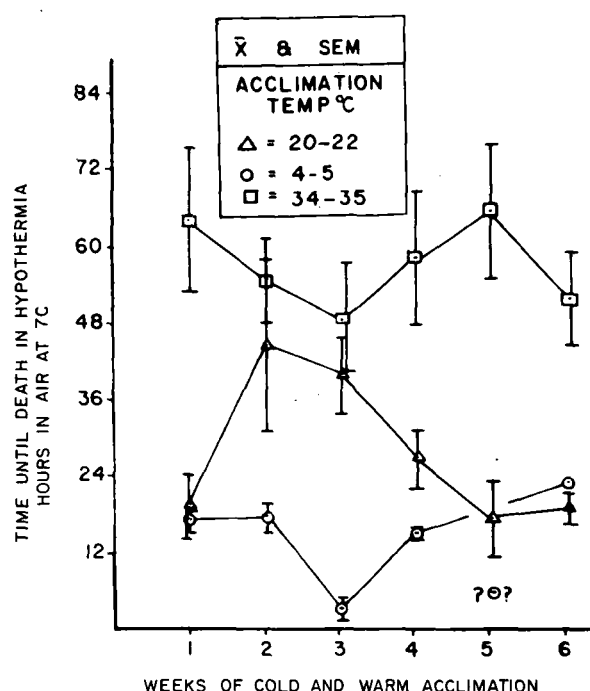
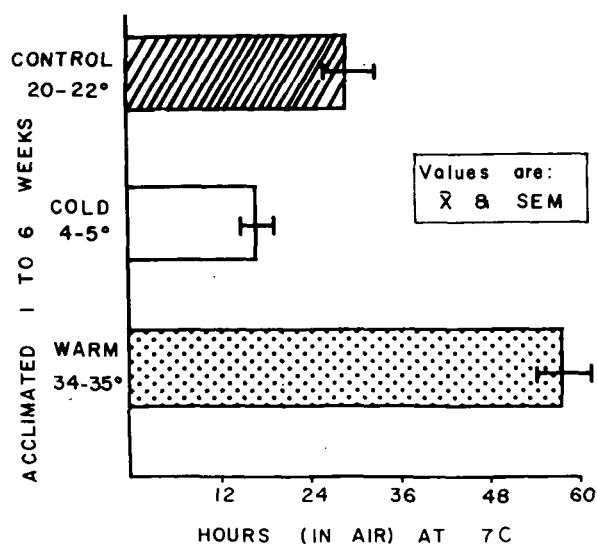FIG. 3. Hypothermic (T_b 7 C) viability of heat- and cold-acclimated hamsters.

FIG. 4. Composite of hypothermic survival (T_b 7 C) for 1- to 6-week heat- and cold-acclimated hamsters.

clearly evident that the heat-acclimated animals showed the longest survival while in a state of hypothermia and the cold-acclimated subjects showed the shortest survival capacity.

A review of the results suggests that the differences between cold-acclimated and the room temperature-maintained subjects might be greatest at 2-, 3-, and 4-week periods. It is well to recall, however, that during the latter periods, that is to say, 4, 5, and 6 weeks, fewer of the cold-acclimated animals became hypothermic. In fact, at 5 weeks none of the six cold-acclimated animals became hypothermic within the designated 12 hr of helium cold exposure. The composite in Fig. 4 shows more substantially the effect of heat and cold acclimation in hypothermia in the hamster.

DISCUSSION

These experiments clearly showed that heat-acclimated hamsters were less resistant to induction of hypothermia, whereas cold-acclimated animals had increased their resistance. In addition, the heat-acclimated animals showed considerably greater viability in hypothermia. What appears to have taken place is that with increased thermogenesis, the cold-acclimated hamsters increased their resistance to induction into hypothermia when exposed to additional vigorous cold. These results were expected on the basis of available knowledge concerning cold adaption. For example, Le Blanc (7) has shown that in rats there is an improved survival at very low temperatures and resistance to hypothermia if they are first exposed to cold, with either continuous or intermittent exposures. Ferguson and Folk (4) showed that cold acclimation of the white rat, house mouse, deer mouse, and collared lemming increased their cold tolerance to exposures of −40 C.

Survival in hypothermia may be a function of the length of time it takes to become hypothermic and, from present results, the altered induction appears to be the key to survival in hypothermia. However, one question which cannot be overlooked is whether hypothermic viability would be extended if the induction time were shortened in cold-acclimated animals.

It is reasonable to assume that induction of hypothermia, by any of the usual methods of cold exposure (e.g., ice-water immersion, wet-fur-plus-cold exposure, hypercapnic-hypoxia-plus-cold exposure) will cause an animal some amount of biological insult, particularly during initial attempts to thermoregulate while exposed to severe cold and later in attempting to maintain functional integrity while at low body temperature. Induction of hypothermia by the helium-cold method (5) undoubtedly causes some amount of stress. Overall, the degree or amount of biological insult is difficult to quantitate. However, the measurable weight loss during induction, about 5 % of body weight, is a tangible parameter which gives some indication of metabolic expenditure. During induction of hypothermia, body weight loss can be credited to at least two functions; excretion (including water losses in urine, ventilation, and defecation) and with the energy expenditures of thermoregulation (shivering and nonshivering thermogenesis). One hypothesis, which the present research supports, is that a shortened induction time results in a reduction in overall stress responses. The fact that heat-acclimated animals lost the least weight and also were the quickest to become hypothermic supports the view that these undergo the least biological insult. In contrast, the cold-acclimated hamsters required the longest period to become hypothermic and, concomitantly, showed the greatest weight loss. The weight loss appears to be a function of the time it takes to undergo hypothermia. In addition, it must be considered that the energy stores dissipated during the lengthy induction period in cold-acclimated hamsters might be severe enough to limit hypothermic viability.

Thus, in the hamster cold acclimation to 4–5 C may turn out to be protective in the event of additional vigorous cold exposure, but is detrimental if the animal is intended for studies of depressed metabolism during hypothermia. This was readily seen in their resistance to hypothermia and their relatively shortened survival while in a state of hypothermia.

The comparative physiological aspect of helium-cold

hypothermia offers several points of interest. Zimin and Runkov (13) have also utilized the cooling action of helium 79% and oxygen 21% at environmental temperatures of 10–13 C and induced hypothermia in albino mice. In comparison with the hamster (Fig. 3) longevity of hypothermic torpor is limited in mice, about 5 hr until death. According to Zimin and Runkov (13) the fall in body temperature in albino mice required about 3 hr to reach approximately 20 C. In our laboratory using the helium-cold method it takes about 1 hr for C3H mice to undergo hypothermia to body temperatures of 13–17 C and survival to 8 hr is frequent (unpublished observations). The further utilization of helium-cold techniques to induce hypothermia in a wider variety of animals seems warrented, particularly in the exploration of factors implicated in hypothermic survival.

It is reasonable to say that numerous experimental approaches have been used in attempting to bring about artificial hibernation, a subject reviewed by Kayser (6), and depressed metabolic states in one form or another. On the

basis of induction of hypothermia for periods of 24, 48, and as long as 72 hr, we suggest that the helium-cold method may well provide the vehicle for production of artificial hibernation. Among the numerous similarities is the depression in cardiovascular and respiratory functions (1, 5), the similarity of blood gas modifications, and blood pH in hibernating and hypothermic hamsters, respectively (8, 12).

The results herein reported on hamsters clearly demonstrate that heat and cold acclimation affect induction into hypothermia and the state of viability in hypothermia. In addition, these experiments present a method for facilitating the depressed metabolism seen in helium-cold hypothermia.

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SEASONAL CHANGES IN THE FATTY ACID SPECTRUM IN THE HIBERNATING AND NON-HIBERNATING GROUND SQUIRREL *CITELLUS TRIDECEMLINEATUS*

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Abstract—1. The total lipid, water content and fatty acid spectrum of blood serum, liver and brown adipose tissue (BAT) were analyzed in hibernating and non-hibernating ground squirrels during an annual cycle.
2. Fatty acids, chiefly oleic and linoleic acids, showed oscillatory cycles in the three tissues studied; these oscillations were less evident in hibernating animals.
3. Brown adipose tissue contained the greatest amount of oleic acid in hibernating animals (60 per cent in March–April). In contrast to serum and liver, docosohexaenoic acid (22:6) was undetectable in BAT.

INTRODUCTION

STUDIES of changes in lipid content in tissues of hibernators have been made at different times of the year (summer, fall and/or winter) and when animals were in hibernation or aroused from hibernation (Musacchia & Wilber, 1952; Galster & Morrison, 1966; Burlington *et al.*, 1969). The present study differs in that ground squirrels, *Citellus tridecemlineatus*, were examined over an annual period. A comparison was made between animals induced to hibernate, T_b 7°C, and others which were not permitted to hibernate. Liver, brown adipose tissue and blood serum were collected from hibernating and non-hibernating animals on a monthly basis and in each tissue a fatty acid (FA) spectrum was determined with gas–liquid chromatography (GLC). In this way, ten fatty acids were separated and followed throughout an annual cycle for hibernating and non-hibernating (control) squirrels. Furthermore, because there appears to be a difference in the FA spectrum between males and females, the data were separated according to sex.

Since much of the chemistry of intermediary metabolism of hibernation is still very incomplete, studies of this kind are expected to lead to information regarding the specific fatty acids which become metabolically dominant in a particular season of the year. These studies might also serve to identify cycling of fatty acids which can then be correlated with other hibernating characteristics. Furthermore, the ground squirrel is now commonly employed in a variety of laboratories and our data show that winter non-hibernating animals must be considered different than

summer non-hibernating animals. This is particularly true with regards to lipid metabolism, and undoubtedly true for other metabolic studies.

MATERIALS AND METHODS

Citellus tridecemlineatus was selected because of its widespread use in laboratories concerned with the biology of hibernation, its availability and ease in inducing hibernation. Collections were made in eastern Kansas in spring and early summer, 1968. In the laboratory they were maintained on an *ad lib.* diet of Wayne Lab Blox and water, and a weekly supplement of lettuce. Thirty squirrels, eighteen males and twelve females, were induced to hibernate by placing them in the cold at 5–7°C from 1 to 2 weeks before use. Only animals that remained in hibernating torpor 4–6 days were used for analysis. Forty-one squirrels, nineteen males and twenty-two females, were used for controls. These were maintained at a room temperature of $23 \pm 1°C$ with a light cycle of 12 hr dark and 12 hr light. Control animals were weighed and then anesthetized with nembutal, 32 mg/kg, prior to withdrawing blood by direct heart stab. Hibernating animals were administered a cervical fracture. Subsequently, for each animal two samples of liver were removed: one for determination of total lipids and lipid analysis by gas chromatography; the other liver sample was used for determination of water content. The latter was performed by drying overnight in a vacuum at 60°C. Samples of scapular brown adipose tissue were also removed and similarly treated. Total fat in both of these tissues is expressed on a dry tissue weight basis.

Total fatty acids were extracted from liver, brown adipose tissue and blood serum according to the method of Lasker & Theilacker (1962) with modifications by Patton & Platner (1970). Methylation of the fatty acids was carried out by the boron trifluoride method of Metcalfe & Schmitz (1961).

A Barber–Colman gas chromatograph was used to separate and quantitate the fatty acids. This instrument was equipped with dual hydrogen flame detectors and dual 8 ft glass columns 0·125 in. in internal dia. The columns were packed with Gas-Pak Wab, 60–80 mesh, coated with 20% ethylene glycol succinate. All analyses were carried out isothermically at 190°C using helium as the carrier gas at a flow rate of 80 ml/min.

Qualitative analysis of the fatty acids was done by comparison to known methylated standards obtained from the National Institutes of Health or purchased from commercial sources. The "carbon number" was obtained by plotting the log of the retention time against the carbon number as described by Woodford & van Gent (1960).

Quantitation of the fatty acids was performed using the model 205 disc-chart integrator. Individual fatty acids are expressed in area percentage of the total peaks being considered. Only fatty acids from 14 to 22 carbons in chain length were considered for data presentation because 96 per cent of animal fatty acids fall in this group. The "new" modified system for naming fatty acids has been used, where both the number of carbons and double bonds in the chain are shown. Thus, myristic is 14:0, myristoleic 14:1, palmitic 16:0, palmitoleic 16:1, stearic 18:0, oleic 18:1, linoleic 18:2, linolenic 18:3, arachidonic 20:4 and docosahexaenoic 22:6.

RESULTS

Body weight of the male hibernating ground squirrels was 18 per cent lower than controls but no significant difference was found between female hibernators and controls. This loss in body weight during hibernation does not appear to reflect a loss of water in either liver or brown adipose tissue (Table 1), since liver remains unchanged and brown fat actually increases in water content.

TABLE 1—PERCENTAGE WATER IN LIVER AND BROWN ADIPOSE TISSUE OF GROUND SQUIRRELS

	Control	Hibernating	Significance: control vs. hibernating
Liver			
Males	65·39 (15)	66·21 (16)	N.S.
	>N.S.	>N.S.	
Females	66·02 (23)	64·00 (13)	N.S.
Brown adipose tissue			
Males	44·4 ± 1·5 (16)	48·9 ± 1·6 (15)	$P = 0·05$
	>N.S.	>N.S.	
Females	47·0 ± 1·2 (22)	51·2 ± 1·1 (11)	$P = 0·02$

Total fatty acid data collected for the entire year and separated according to sex into control and hibernating ground squirrels are presented in Table 2. Neither brown adipose tissue nor serum from hibernating animals was significantly different from the controls of either sex. However the liver total fatty acids of the hibernators increased by 50 per cent in both males and females.

TABLE 2—TISSUE TOTAL FATTY ACIDS (g/100 g dry wt.) IN HIBERNATING AND NON-HIBERNATING GROUND SQUIRRELS

	Control	Hibernating	Significance: control vs. hibernating
Blood serum			
Males	709·0 ± 173 (16)	592·3 ± 74·9 (16)	N.S.
	>N.S.	>N.S.	
Females	533·7 ± 71·5 (22)	581·3 ± 88·1 (13)	N.S.
Brown adipose tissue			
Males	43·6 ± 3·18 (16)	39·5 ± 3·23 (18)	N.S.
	>N.S.	>N.S.	
Females	40·2 ± 3·68 (22)	41·5 ± 3·26 (12)	N.S.
Liver			
Males	8·2 ± 0·95 (17)	16·2 ± 2·00 (17)	$P = 0·001$
	>N.S.	>N.S.	
Females	8·7 ± 0·78 (22)	18·5 ± 2·64 (13)	$P = 0·002$

Serum

The serum fatty acid spectrum showing yearly averages for control and hibernating male and female ground squirrels is presented in Table 3. Two fatty

TABLE 3—SERUM FATTY ACIDS OF MALE AND FEMALE GROUND SQUIRRELS

Fatty acid	Control males	Hibernating males	Significance	Control females	Hibernating females	Significance
14:0	1·29 ± 0·22 (19)*	0·97 ± 0·14 (16)	N.S.	1·22 ± 0·18 (20)	0·61 ± 0·09 (13)	P = 0·004
14:1	0·36 ± 0·07 (18)	0·43 ± 0·79 (14)	N.S.	0·59 ± 0·12 (18)	0·37 ± 0·07 (13)	N.S.
16:0	21·04 ± 0·96 (20)	19·84 ± 0·85 (15)	N.S.	19·96 ± 0·86 (20)	18·75 ± 0·84 (13)	N.S.
16:1	3·17 ± 0·47 (20)	3·00 ± 1·54 (16)	N.S.	3·34 ± 0·43 (20)	3·20 ± 0·30 (13)	N.S.
18:0	9·86 ± 0·93 (20)	9·02 ± 0·52 (16)	N.S.	7·81 ± 0·57 (20)	9·81 ± 0·59 (13)	P = 0·016
18:1	29·86 ± 2·99 (20)	25·50 ± 2·49 (16)	N.S.	29·11 ± 2·45 (20)	22·74 ± 1·20 (13)	P = 0·021
18:2	27·98 ± 2·28 (20)	31·65 ± 1·36 (16)	N.S.	28·69 ± 1·78 (20)	35·38 ± 1·23 (13)	P = 0·003
18:3	0·77 ± 0·17 (20)	0·61 ± 0·18 (16)	N.S.	0·92 ± 0·15 (20)	0·29 ± 0·06 (13)	P = 0·001
20:4	3·33 ± 0·61 (20)	5·11 ± 0·51 (16)	P = 0·028	4·72 ± 0·83 (20)	5·59 ± 0·48 (13)	N.S.
22:6	1·53 ± 0·32 (15)	3·29 ± 0·54 (16)	P = 0·007	1·53 ± 0·39 (18)	2·28 ± 0·48 (12)	N.S.

* Figures in parentheses indicate number of animals.
± = Standard error of the mean.   N.S. = Not significant.   P = Probability.

acids in serum, arachidonic and docosahexaenoic, were significantly increased in hibernating males. The serum in hibernating females showed decreases in myristic, oleic and linolenic while stearic and linoleic increased. A net increase in hibernators appears to favor the saturated fatty acids by about 1·0 per cent for the females whereas the male squirrels show an increase of 3·5 per cent in unsaturated fatty acids. These changes were calculated only for the fatty acids that showed a statistically significant difference between controls and hibernators on an annual basis. Individual monthly changes are given (Fig. 1) and considerable variation in serum fatty acids during the year can be seen. The greatest variation appears in oleic and linoleic acids which range from 15 to 54 per cent respectively, with an increase in the fall and winter, and a decrease in late spring and summer in both male and female squirrels.
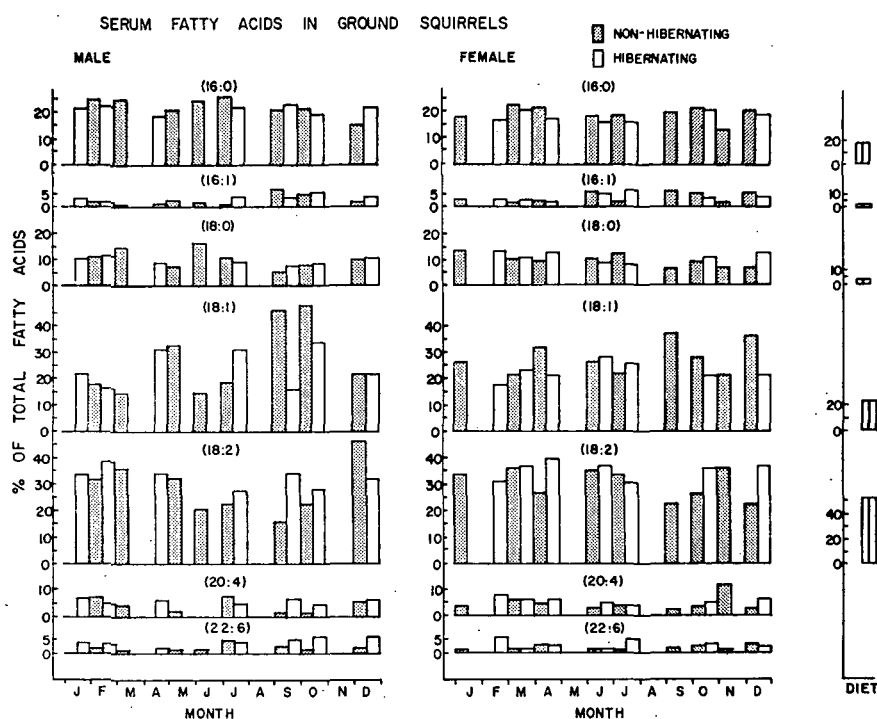

FIG. 1. Variations in serum fatty acids in ground squirrels during an annual cycle. Hibernating $T_b = 7°C$ and non-hibernating "controls" are normothermic, $T_b = ca.$ 37°C. Fatty acid composition of diet is also given.

Myristoleic, palmitic and palmitoleic acids remain uniform throughout the year in both control and hibernating ground squirrels. Interconversion of palmitic to stearic by the chain elongation system does not show relevance since both of the fatty acids appear to vary independently.

Oleic acid shows the most striking change in control subjects, it oscillates throughout the seasons, 18 per cent in February, 14 per cent in March and 32 per cent in May. In September and October it exceeds 50 per cent, then declines to 21 per cent in December. The hibernating subjects do not show these great oscillations. The rise of oleic acid in hibernators in April coincides with control animals, remains elevated in July, falls in September and rises to about 30 per cent in late fall, and in winter declines along with the controls. Linoleic acid in control animals remains elevated at 32–36 per cent until June at which time it drops to 21 per cent, and 15 per cent in September, with a gradual increase to 46 per cent in December. The hibernators show smaller oscillations of linoleic throughout the year than do the controls.

Brown adipose tissue (BAT)

Oleic acid appears to be elevated in hibernating ground squirrels, 54 per cent compared to 44 per cent for controls as yearly averages (Table 4). The non-hibernating animals show considerable variation from month to month (Fig. 2).

Palmitic acid in brown adipose tissue of control animals ranges from 13 per cent in March to 20 per cent in September whereas the range for hibernators is from 8·4 per cent in April to 16·1 per cent in September. The average for the year was 16·0 per cent for control animals compared to 11·1 per cent for hibernators. It appears that male hibernators have less palmitic acid ($P = 0.001$) in brown adipose tissue. Saturated fatty acids (14:0, 16:0, 18:0) in brown adipose tissue total 23·8 per cent for control animals and 16·3 per cent for hibernators. Thus hibernators have 7·5 per cent less saturated FA than control animals. The unsaturated fatty acids (18:1, 18:2, 18:3, 20:4) in brown adipose tissue in hibernators total 83·4 per cent compared to 75·5 per cent for control animals, thus hibernating ground squirrels have 7·9 per cent more unsaturated fats than the control group.

Linoleic acid variations are minimal in brown adipose tissue of male hibernators, the lowest value, 23·0 per cent, occurring in October. Control animals show an oscillatory pattern with a high of 45 per cent in March and a low value of 13 per cent in September. Female squirrels show a pattern similar to male animals except that linoleic acid values were generally 3 to 4 per cent higher (Table 4). In the females linoleic acid decreased in brown adipose tissue but increased in liver in both males and females.

Arachidonic acid is a very small percentage of brown adipose tissue. Male hibernators have slightly more arachidonic acid than controls (1·0 vs. 0·45 per cent, $P = 0.003$). The same relationship does not obtain in the females. The largest amount of arachidonic acid appears in hibernators and non-hibernating animals in winter months.

The major fatty acid changes in brown adipose tissue (Table 4) occurring in hibernators resemble those in liver (Table 5) for both male and female squirrels. These changes have one common pattern which seems to characterize the liver

TABLE 4—BROWN ADIPOSE TISSUE FATTY ACIDS OF MALE AND FEMALE GROUND SQUIRRELS

Fatty acid	Control males	Hibernating males	Significance	Control females	Hibernating females	Significance
14:0	0·85±0·13 (18)	0·59±0·09 (17)	N.S.	0·68±0·10 (23)	0·43±0·10 (13)	N.S.
14:1	0·10±0·03 (18)	0·27±0·07 (16)	P = 0·045	0·36±0·14 (23)	0·24±0·08 (13)	N.S.
16:0	16·70±0·90 (18)	10·80±0·57 (18)	P = 0·001	15·50±0·69 (23)	11·70±0·99 (13)	P = 0·003
16:1	2·85±0·30 (18)	3·36±0·32 (16)	N.S.	2·44±0·25 (23)	3·23±0·34 (13)	N.S.
18:0	6·27±0·26 (18)	4·98±0·24 (17)	P = 0·001	6·63±0·26 (23)	5·37±0·55 (13)	P = 0·045
18:1	44·23±1·69 (18)	54·54±1·69 (17)	P <0·001	40·67±1·40 (23)	51·03±1·66 (13)	P <0·001
18:2	26·30±2·29 (18)	22·80±1·11 (17)	N.S.	30·00±1·99 (23)	25·10±1·37 (13)	P = 0·045
18:3	1·60±0·12 (18)	1·50±0·10 (17)	N.S.	2·00±0·18 (23)	1·40±0·14 (13)	P = 0·009
20:4	0·45±0·12 (18)	1·00±0·15 (17)	P = 0·003	1·18±0·20 (23)	0·98±0·18 (13)	N.S.
22:6	Trace	Trace		Trace	Trace	

Trace = <0·1 per cent.

TABLE 5—LIVER FATTY ACIDS OF MALE AND FEMALE GROUND SQUIRRELS

Fatty acid	Control males	Hibernating males	Significance	Control females	Hibernating females	Significance
14:0	1·37±0·20 (21)	0·57±0·14 (17)	P = 0·001	1·28±0·16 (21)	0·81±0·15 (13)	P = 0·045
14:1	0·38±0·06 (21)	0·48±0·13 (17)	N.S.	0·29±0·04 (21)	0·49±0·13 (13)	N.S.
16:0	21·48±0·77 (21)	16·42±0·84 (17)	P = <0·001	21·10±0·73 (21)	14·49±0·55 (13)	P = 0·001
16:1	3·57±0·41 (21)	3·81±0·55 (16)	N.S.	3·27±0·39 (21)	4·83±0·67 (13)	P = 0·045
18:0	11·28±0·74 (21)	7·84±0·80 (17)	P = 0·002	10·18±0·87 (21)	7·05±1·15 (13)	P = 0·036
18:1	32·12±3·18 (21)	36·28±2·29 (17)	N.S.	29·33±2·58 (21)	37·49±2·25 (13)	P = 0·021
18:2	18·35±1·32 (21)	25·04±1·13 (17)	P = 0·001	23·08±1·52 (21)	27·92±1·43 (13)	P = 0·021
18:3	0·66±0·17 (21)	1·18±0·26 (17)	N.S.	0·89±0·14 (21)	0·79±0·15 (13)	N.S.
20:4	5·40±0·67 (21)	4·74±0·66 (16)	N.S.	6·57±0·82 (21)	3·94±0·78 (13)	P = 0·21
22:6	4·12±0·57 (21)	3·24±0·58 (17)	N.S.	4·00±0·67 (19)	2·71±0·40 (11)	N.S.

* Figures in parentheses indicate number of animals.
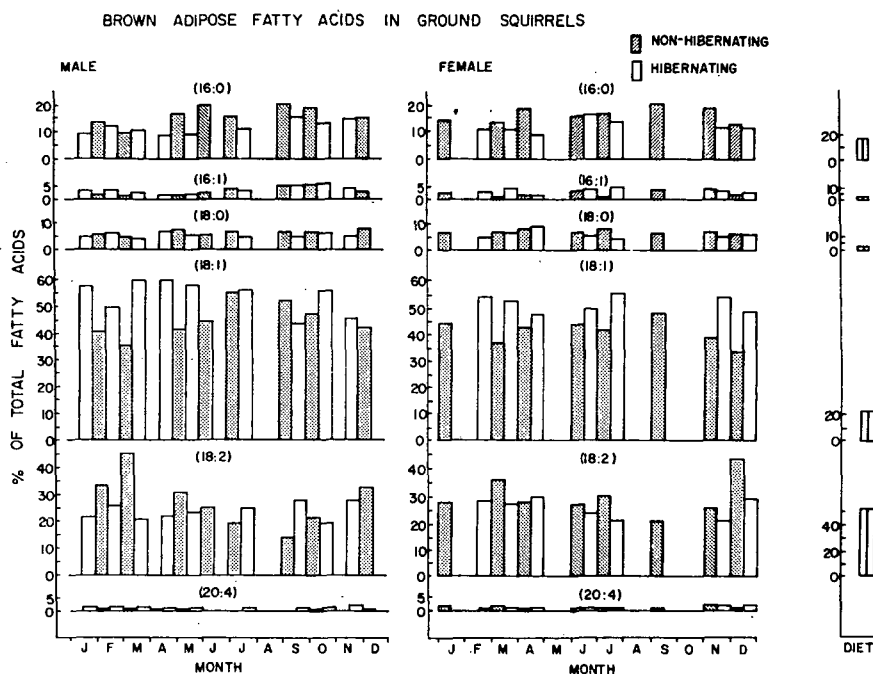± = Standard error of the mean.   N.S. = Not significant.   P = Probability.

FIG. 2. Variations in brown adipose tissue, fatty acids in ground squirrels during an annual cycle. Hibernating $T_b = 7°C$ and non-hibernating "controls" are normothermic, $T_b = ca.$ 37°C. Fatty acid composition of diet is also given.

and brown adipose tissue in hibernating squirrels, namely, palmitic and stearic acids decrease and oleic acid increases.

Liver

In general, palmitic acid in the liver is depressed in hibernating males. The yearly average is 15·8 per cent and ranges from 11·0 per cent in January to 16·0 per cent in April. Female squirrels follow a pattern almost identical to the male squirrels. Seasonal oscillations in palmitic acid appear to diminish in hibernating squirrels (Fig. 3). Stearic acid is also depressed in both male and female hibernators particularly during the winter months (Fig. 3). The two major saturated fatty acids, palmitic and stearic, are generally lower in hibernating male and female animals (Table 5 and Fig. 3).

Oleic acid shows a pattern of oscillation in control animals, becoming elevated in June and July. The lower values occur in February and April and again in October and December, the highest value, 53 per cent, was found in July in males and females and September in males. Yearly averages, 37·0 per cent for hibernators and 30·1 per cent for controls, were significantly different ($P = 0·02$) when both male and female data were pooled.
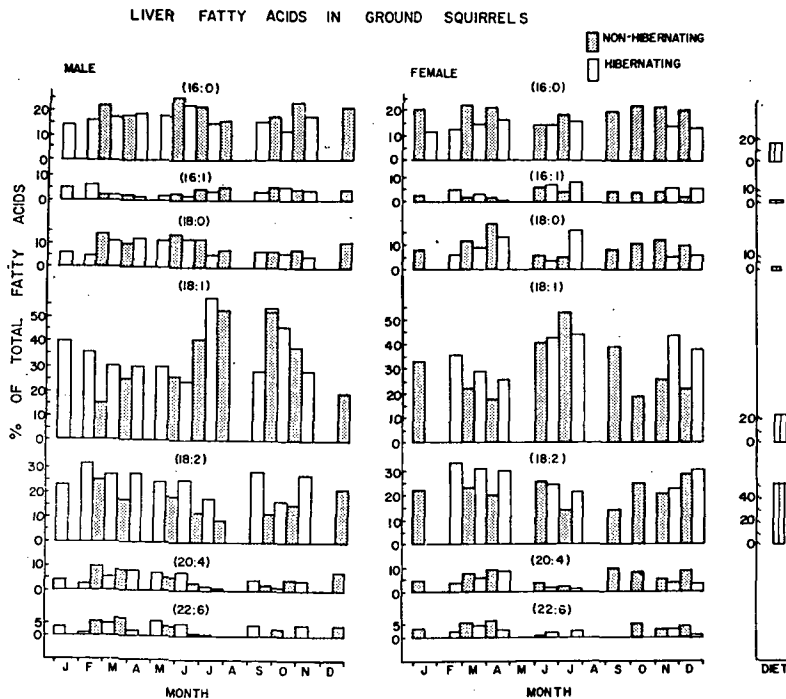
FIG. 3. Variations in liver fatty acids in ground squirrels during an annual cycle. Hibernating $T_b = 7°C$ and non-hibernating "controls" are normothermic, $T_b = ca.$ 37°C. Fatty acid composition of diet is also given.

Linoleic acid is significantly elevated in both male and female hibernators (Table 5). A definite trend shows the low point to be in midsummer and early fall (July and September), a result which is clearly opposite to that found for oleic acid.

Arachidonic acid is present in liver (and serum) in amounts 4–6 per cent higher than in brown fat. Livers of female hibernating animals contained their lowest amount, 1·5 per cent, in June and July and the highest amount, 8·0 per cent, in April. The yearly average for hibernating squirrels was 4·0 per cent compared to 6·5 per cent for the controls. Of all the unsaturated fatty acids (14:1, 16:1, 18:1, 18:2, 18:3) arachidonic (20:4) generally was found to be significantly lower in liver of hibernating females. Liver fatty acid data presented in Table 5 show a net increase of 6·69 per cent unsaturation in the male hibernators and 11·93 per cent in the female hibernators.

DISCUSSION

The lower body weight in the hibernating squirrels was not unexpected since these animals are known to gain considerable weight prior to hibernation and to

utilize stored fat during hibernation (Howell, 1938; Kayser, 1961); this leads to an overall loss in body weight. Seasonal changes in water content in the whole carcass of *Citellus lateralis* has been studied by Jameson & Mead (1964). Their data show a decline in water and an increase in fat just prior to hibernation. The present study indicates that water content in the liver of hibernating ground squirrels remains unaltered and the water content increased in brown adipose tissue. Similar results have been reported for the cold acclimated white rat (Shields, 1962).

One of the most striking features noted in the fatty acid concentration of the three tissues studied was the reduced seasonal oscillatory pattern of hibernating squirrels. This was most pronounced for oleic and linoleic acids in serum and liver and least in brown fat. These seasonal alterations were not as pronounced for palmitic or stearic acids. The fatty acid patterns in the control animals show large seasonal changes in the unsaturated fatty acids as early as June or July. These animals appear to respond to the approach of the fall season without known temperature, visual or food cues since all were on the same ambient temperature, light cycle and diet. The partial loss of these oscillations during hibernation is probably related to a depression of enzymes responsible for synthesis or degradation of fatty acids at seasonal intervals.

Brown adipose tissue showed a significant increase in water content in both male and female hibernating squirrels. Such an increase in water content was reported previously for the 13-lined ground squirrels by Burlington *et al.* (1969). These authors also reported an increase in total lipid of brown fat of the hibernating ground squirrel whereas our results showed no change in total fatty acids in either male or female animals. This contradiction may be due to the different methods of extraction and calculation of results. Their tissue lipid weight was based on wet tissue weight whereas ours was calculated on a dry tissue weight basis. Furthermore our extract represents total fatty acids whereas theirs represents total lipid presumably including cholesterol and other lipids. Our results, however, agree with Pagé & Babineau (1950) who showed no increase in total lipid content of brown adipose tissue of rats following cold acclimation. That BAT is involved in heat production during arousal from hibernation has been well established (Smith & Hock, 1963; Brück, 1970; Joel, 1970), but its seasonal cyclic fatty acid composition has not been previously described.

It is apparent that in liver and brown adipose tissue the major fatty acid is oleic and it is found in statistically greater amounts in hibernating squirrels. Linoleic acid is present in the next largest amount and is also found in greater concentration in liver of hibernators than in control animals. It becomes apparent from Fig. 2 that in BAT as linoleic acid increases, oleic acid decreases. Why these are inversely related is not entirely clear. The fatty acid composition of the diet contained 48·07% linoleic acid and 21·08% oleic acid. It is known that the mammalian organism cannot synthesize linoleic acid but since ample amounts were provided in the diet, conversion to a host of polyunsaturated acids can occur, (i.e. to docosahexaenoic acid). However, this did not occur in brown

adipose tissue for only trace amounts of docosahexaenoic (22:6) were present. Similarly oleic acid can be converted to icosatrienoic acid (20:3) by means of the chain elongation enzyme system from palmitoyl-CoA. Without radioactive studies these conversions can only be stated as speculations.

SUMMARY

The thirteen-lined ground squirrel was studied under controlled laboratory conditions including hibernation on a monthly basis, for one annual period. In most instances hibernating and non-hibernating (control) animals were sacrificed each month and samples of serum, liver and brown adipose tissue were taken for analysis of total fatty acid, water content and fatty acid spectrum. The male and female hibernating animals showed an increase in total lipids of the liver but not in serum or brown adipose tissue. Water content of brown adipose tissue increased approximately 4·5 per cent for both sexes. The dominant fatty acids in all three tissues as determined by gas chromatography were palmitic, oleic and linoleic.

The two polyunsaturated fatty acids, arachidonic and docosahexaenoic were significantly increased in serum of male hibernating subjects and arachidonic acid was significantly reduced in liver of female hibernators.

Several of the fatty acids exhibited an oscillatory cycle most noticeable in the control animals and dampened in the hibernators. These oscillations were most prominent for oleic and linoleic acids. The liver showed changes similar to serum except that oleic and linoleic acids appear to be inversely related. In liver tissue, high values for oleic appeared in summer months and conversely low values for linoleic were reached at this time.

Brown adipose tissue contained the greatest amount of oleic acid in hibernating animals reaching 60 per cent in March–April in male animals and slightly less in females. In control, non-hibernating squirrels, linoleic and oleic acids followed the inverse pattern in BAT. Docosahexaenoic acid was undetectable in BAT.

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Evidence for a Metabolic Limitation of Survival in Hypothermic Hamsters[1]
(36658)

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The survival of homeotherms in profound hypothermia has been investigated for many years. There are two main factors which contribute to the ultimate demise of the hypothermic animal: cardiac failure or respiratory failure, both resulting in an anoxic death of the tissues (1). The mechanisms leading to death are highly dependent upon inherent physiological characteristics of the animal itself and the temperature at which it is maintained. Adolph *et al.* (2) have shown that respiration ceases at a higher temperature than the beating of the heart in hypothermic rats, and Fisher *et al.* (3) have shown that a decline in cardiac output is the primary failure in dogs maintained at 23°.

The hypothermic hamster (*Mesocricetus auratus*) is a special case. This animal has the ability to hibernate for periods as long as seven days (4), but will last for approximately 22 hr in helium-cold induced hypothermia at a rectal temperature of 7°. The underlying factor(s) limiting survival in the hypothermic state is the subject of this paper.

Anderson *et al.* (5) have shown that the heart rate and oxygen consumption are stable for several hours in the hypothermic hamster. During this stable period the heart is not subjected to an anoxic stress (6).

Musacchia (7) has shown that cold or heat acclimation prior to the induction of hypothermia affects induction time and survival time in hypothermia. Heat acclimation will shorten induction time and prolong the survival time while cold acclimation has the opposite effect. However, the animals shiver during the induction period thus expending a considerable amount of energy in an attempt to remain homeothermic. If this energy loss has an effect on the survival time in hypothermia it may explain the discrepancy between the heat-acclimated and cold-acclimated hamsters as well as the hypothermic and hibernating hamsters. Thus, an attempt was made in this study to investigate the effect of shortened induction time on the energy stores and the survival of the hamster in hypothermia.

The results of this study may also have an application in the interpretation of experiments on nonhibernators using the Giaja technique (8) or other similar stressful methods of induction (2,9).

Methods. Hamsters (*Mesocricetus auratus*) of both sexes were inducted into profound hypothermia, rectal temperature (T_{re}) of 7°, using the method of Fischer and Musacchia (10). Briefly, the animals are placed in a 20% O_2 and 80% He atmosphere at 0° until they reach a T_{re} between 10 and 7°. They are then transferred to air (T_a) at 7° until used.

The first series of experiments involved a group of eleven hamsters which were clipped of their fur under light sodium pentobarbital anesthesia (65 mg/kg ip) 16 hr before induction into hypothermia. The control group of 11 hamsters were also lightly anesthetized at the same time. After induction, all of these animals were kept at T_{re} 7° until death, which was determined by monitoring the ecg (lead II) and visual observations.

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TABLE I. Induction Time and Survival Time in Hypothermia, $T_{re} = 7°$, of Control and Fur-Clipped Hamsters. Values are Means ± SE.

	Induction time (hr)	Survival time (hr)
Control (n = 11)	7.8 ± 0.7	22.0 ± 2.9
Fur-clipped (n = 11)	3.8 ± 0.3 $p < 0.001$	38.7 ± 4.8 $p < 0.01$

A second experiment involved two groups of hamsters; animals in both groups were inducted into hypothermia as described above. Each group contained subgroups of fur clipped and control hamsters. Blood was taken from one group of hamsters immediately following induction. Hamsters in the other group were allowed to remain at T_{re} 7° until the onset of gasping before the blood samples were drawn. Gasping indicates the failure of spontaneous respiration and precedes death by approximately two hr (5). The blood samples were centrifuged and the plasma analyzed for glucose colorimetrically by means of a glucose oxidation reaction using the Glucostat preparations (Worthington Biochemical Corporation, Freehold, NJ.)

A third experiment followed the same procedure as the second except that instead of taking blood samples, a section of the liver was removed and immediately frozen with dry ice. The liver was then homogenized in 10% trichloroacetic acid, filtered, and an aliquot of the filtrate was analyzed for glycogen according to the method of Carroll, Longley, and Roe (11).

Results. The reduction of insulation by clipping the fur from the hamsters resulted in an induction time less than half of that of the control hamsters (Table I). Survival under hypothermic conditions was significantly prolonged when induction time was shortened.

Table II contains the results of the glucose and glycogen analyses. The fur-clipped hamsters had a significantly higher plasma glucose immediately following induction into hypothermia, but at the onset of gasping the plasma glucose levels in animals in both groups were the same.

Similar results were found with the liver glycogen study. The control hamsters depleted more of their liver glycogen during the induction period than the fur-clipped hamsters. At the onset of gasping, animals in both groups were depleted of liver glycogen, but the fur-clipped hamsters were significantly lower than the controls. The gasping control animals had higher liver glycogen levels than control animals immediately following induction.

Discussion. The prolongation of survival in hypothermia by shortening the induction time as demonstrated in these experiments is a new concept in the study of hypothermia. Popovic (12) varied the induction time of rats using several techniques but found no difference in survival times. Instead the survival of the rats was related to the temperature at which they were maintained in hypothermia, the lower the temperature the shorter the survival time. This suggests that there may be a significantly different mechanism leading to failure in the hamster as compared to the rat or else Popovic's different techniques (12) did not alter the stress of induction to a significant degree.

The analyses of glucose and glycogen indicate that the survival of the hamster may be related to the depletion of energy stores dur-

TABLE II. Plasma Glucose and Liver Glycogen of Control and Fur-Clipped Hamsters. Hypothermia, $T_{re} = 7°$. Values are Means ± SE.

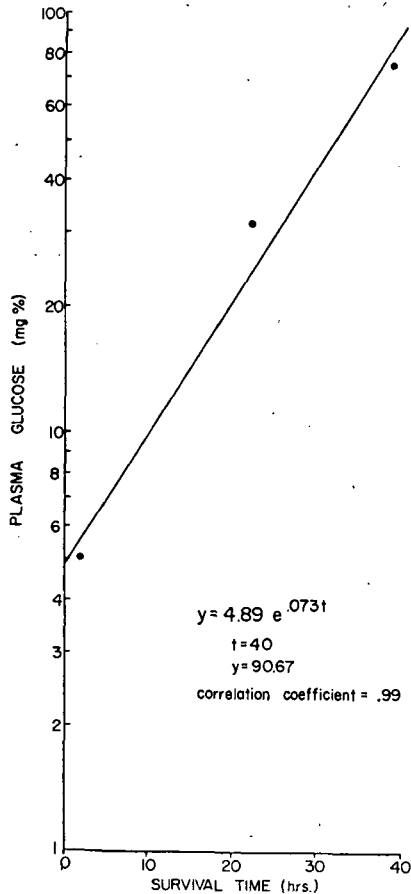|	Immediately following induction	Onset of gasping
Plasma glucose (mg %)		
Control	31.3 ± 6.76 n = 15	5.48 ± 1.73 n = 8
Fur-clipped	73.5 ± 14.7[a] n = 12	4.65 ± 2.32 n = 6
Liver glycogen (mg/g wet weight)		
Control	.422 ± .11 n = 9	.869 ± .14 n = 8
Fur-clipped	10.6 ± 2.17[a] n = 10	.196 ± .003[a] n = 5

[a] Significantly different from controls ($a = .05$).

FIG. 1. Survival in hypothermia, T_{re} 7°, as a function of plasma glucose concentration. Average values (●) are used to determine the best fit line.

ing the induction period. The longer the period of stress during induction the greater will be the depletion of carbohydrate stores with a resultant decrease in survival in hypothermia.

The relation between plasma glucose and survival can be easily seen by combining the data from the survival experiment and the glucose experiments in the graph shown in Fig. 1. In this figure the natural log of the average plasma glucose concentration for each group is plotted as a function of the survival time of the corresponding group in the survival experiments. The glucose levels at gasping for control and fur-clipped animals were combined to give one point. If the average values are used to determine the best fit line

the result is $y = 4.87$ exponent $.073t$, with a correlation coefficient of .99. The average values must be used since the survival times of the individual animals used for glucose determinations were not measured. Thus, an exponential depletion of plasma glucose allows the prediction of survival time in hypothermia simply by measuring the plasma glucose anytime after induction.

The exponential depletion of plasma glucose, seen in this study, may not obtain in situations in which initial values are high. For example, in the hibernating ground squirrel there is a linear decay in plasma glucose from 150 mg% at the onset of a hibernating period to near 50 mg% at the end of the period (13). It seems reasonable to suggest that at low body-temperatures and high plasma glucose levels the metabolism of glucose will depend on enzyme activity. In contrast, at low plasma levels such as we found in the hypothermic hamster, glucose availability might be the rate-limiting factor and result in a first order reaction.

Not only do the fur-clipped hamsters have a higher liver glycogen content immediately following induction than does the control group, but for unknown reasons they also seem to be able to deplete their liver glycogen stores to a lower level before failing. The liver glycogen in the control group tends to be higher at the onset of gasping than immediately following induction. In comparison to the normal liver glycogen levels in these animals this increase in liver glycogen during a hypothermic exposure is very slight. This suggests that the animal's capacity for gluconeogenesis may be limited.

It is seen from the data reported here that the failing organ is extremely dependent upon plasma glucose levels for viability. The brain, including the respiratory centers, is almost completely dependent upon glucose for its source of energy (14). It is also known that when glycolysis and oxygen consumption are blocked, the respiratory center fails before the heart stops beating in neonatal rats (15). Artificial respiration will also prolong survival in hypothermic rats (16).

It appears that the hypothermic hamster maintains a steady state until the depletion

of the carbohydrate stores. The heart rate and oxygen consumption are stable for several hours (5). The homeostatis maintenance of the heart in hypothermia has been well established. Anderson et al. (6) have shown that the heart of the hamster is not subjected to anoxic stress during prolonged hypothermia. The metabolism and membrane potentials are maintained in the hearts of hypothermic ground squirrels (17). It is also known that the heart is not dependent upon glucose for its energy supply, viz., the hypothermic rabbit heart will utilize pyruvate in preference to glucose in the presence of endogenous fatty acids (18).

Although the level of oxygen consumption that is maintained during hypothermia is about half of that of a hibernating hamster (5), this does not mean that the tissues are anoxic. Popovic (19), using the Giaja hypercapnic-hypoxia technique, reported that the hypothermic (10°) winter ground squirrel has an oxygen consumption many times higher than the hypothermic summer animal, without any difference in the survival time. Also Fisher et al. (3) have shown that in hypothermic dogs (23°) a stable oxygen consumption can be maintained in spite of a declining cardiac output by increasing the A–V oxygen difference. This demonstrates that the tissues could extract more oxygen from the blood if it were necessary to do so.

After the relatively stable period during which the liver glycogen and plasma glucose are depleted, the hamster begins a period of abnormal respiration typified by periods of gasping and apnea and finally respiratory arrest (5). Therefore, these data together with previous reports from this laboratory are consistent with the hypothesis that the primary cause of death in the hypothermic hamster is failure of respiration due to a depletion of energy supplies to the respiratory center.

It is of interest to note that this decline in plasma glucose levels may not be an abnormality of hypothermic hamsters as compared to hibernating ones. Galster and Morrison (13) reported that the plasma glucose levels of hibernating arctic ground squirrels also decline and are restored to higher levels during the arousal periods. They suggest that the

decline of carbohydrates in plasma, liver, and muscle may actually initiate the arousal process.

The hypothermic hamster is different only by a matter of degree since it is partially depleted of its carbohydrates at the onset of hypothermia and also because it is unable to initiate the arousal process. If the hamsters are removed from the cold room when the plasma glucose is depleted (onset of gasping) they will rewarm at room temperature and survive with no notable ill effects.

It is probable that the hypothermic hamster would survive for the same length of time that it could hibernate if a means of reducing the body temperature in an unstressful way were possible.

Summary. Two groups of hamsters (Mesocricetus auratus) were inducted into hypothermia by the helium-cold method and survival time was determined. The induction time of one group of hamsters was reduced by clipping the fur prior to the induction procedure. Additional groups of fur-clipped and nonclipped control hamsters were used for determinations of plasma glucose and liver glycogen immediately following induction and at the onset of gasping (approximately 2 hr before death). The fur-clipped hamsters had a shorter induction time, a longer survival time, and higher plasma glucose and liver glycogen immediately following induction. At the onset of gasping both groups had similar, extremely low plasma glucose levels and the fur-clipped animals had lower liver glycogen values. Survival time in hypothermia correlates with the plasma glucose levels which decay exponentially during hypothermia. These findings are consistent with the hypothesis that the primary cause of death in the hypothermic hamster is failure of respiration due to the depletion of carbohydrate energy supplies and may explain why survival time in hypothermia is shorter than the normal hibernation time of the hamster.

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Postirradiation Hibernation and Radiation Response of Ground Squirrels: Telemetry Surveillance

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BARR, R. E., AND MUSACCHIA, X. J., Postirradiation Hibernation and Radiation Response of Ground Squirrels: Telemetry Surveillance. *Radiat. Res.* **51**, 631–637 (1972).

Temperature sensitive radio transmitters were implanted in the abdominal cavity of ground squirrels, *Citellus tridecemlineatus*. A 72-channel telemetry receiver system was constructed to monitor the hibernation patterns, as reflected in body temperature changes, of 72 squirrels given 1250 rads ^{60}Co gamma radiation and 20 nonirradiated controls. Forty-eight squirrels were irradiated while torpid and 24 while active. These groups were equally divided into three postirradiation groups, one group each being maintained for 8 weeks at temperature environments, 5, 13, and 23°C. The results showed the highest levels of survival among squirrels irradiated while torpid and that additional increase in survival may have occurred due to postirradiation hibernation. No significant effect was noted relating to the three postirradiation environments.

INTRODUCTION

Numerous investigations (*1–5*) have been made on the radiation response of hibernators. Comparative responses of seasonal effects (*3*), body temperature at the time of irradiation (*5*), and postirradiation cold exposure (*1, 4, 5*) have been studied. These investigations used percent survival and/or mean survival time as a measure of the radiation response.

The criteria of percent survival at the end of a given period of time and mean survival time are frequently used in radiation studies on homeothermic animals, such as mice and rats. If the same criteria are to be used in radiation studies on hibernators or poikilotherms, experimental protocol should include techniques for assessing the metabolic state of the animals before, during and after irradiation.

631

Body temperature is a useful long-term index of overall metabolic rate since any extended change in metabolic rate would result in a change in body temperature. Thus an indication of when an animal is active, entering into hibernation, hibernating or arousing from hibernation can be obtained by monitoring body temperature.

Implanted temperature sensitive radio transmitters provide an acceptable system for continuously monitoring body temperature over long perids of time in rodents. Such a system allows undisturbed free movement of the animals. In the case of hibernators, temperature monitoring is effected in a way which permits the animals to follow hibernation activity cycles as endogenous control mechanisms and the environment dictate.

This paper presents results obtained from an experiment designed to show relationships between postirradiation hibernation and the radiation response of ground squirrels. These relationships were determined by monitoring postirradiation hibernation activity cycles with a temperature sensitive telemetry system.

MATERIALS AND METHODS

The ground squirrel, *Citellus tridecemlineatus*, was used for this study. The experiment began in early December, at which time the weight of the squirrels ranged from 170 to 290 g. Most of these animals were young of the year and had been trapped in eastern Kansas the previous spring. During initial laboratory caging they were housed four per cage. In late summer, they were separated and housed individually in plastic cages ($26 \times 20 \times 15$ cm) which would be used throughout the experiment. Animal rooms were maintained at 22–24°C and a 12-h light:12-h dark cycle; cold rooms were darkened (24 h dark). Diet consisted of Wayne lab blocks, sunflower seeds and water, *ad libitum*.

To monitor body temperature continuously, small, implantable temperature sensitive radio transmitters were constructed. The details of these transmitters are given elsewhere (6). By means of a laparotomy, transmitters were placed free-floating in the abdominal cavity of squirrels. A total of 92 squirrels were used in the experiment. Three to four weeks following implantation, 72 squirrels were given 1250 rads of ^{60}Co radiation, 24 with body temperatures between 5.5 and 8.5°C, 24 with body temperature between 12.5 and 16.0°C, and 24 normothermic animals with body temperature near 37°C. As controls, 20 nonirradiated squirrels were used to assess the effects of transmitter implantation.

After irradiation each transmitter was checked against its calibration curve by comparing its response to the animal's temperature obtained with a rectal thermometer. Following this procedure each squirrel was individually housed in its plastic cage which was then placed on a pair of receiving antennas of the recording system. Eight squirrels from each irradiated group were maintained in each of three temperature environments, 5, 13, and 23°C, for 7–8 weeks. Control squirrels were also placed in the three environments, seven at 5 and 13°C and six at 23°C, at this time.

During this period body temperature was recorded continuously by means of the transmitted radio signals. In this way hibernation activity was monitored and survival time was obtained. Food and water were provided *ad libitum*.

The recording system had 72 receiving channels. Data was recorded on an Esterline–Angus 24-channel chart recorder and on magnetic tape. Details of the receiving and recording system are described elsewhere (6). Twenty-four channels were allocated to each of the three temperature environments mentioned above. At first only the 72 irradiated squirrels were placed on receiving stations. As some of these animals died the telemetry position was filled by control squirrels. At the end of 8 weeks after the first squirrel was irradiated, all squirrels were put in a 23°C environment and body temperature monitoring was terminated. Rectal temperatures were taken at this time to check transmitter function.

RESULTS

Percent survival within each group is indicated in Table I. Each block in the matrix is divided into two parts; numbers in the upper parts give percent survival at the end of 8 weeks in the various postirradiation environments, while numbers in the lower parts are percent survival at 90 days postirradiation, i.e., at 8 weeks plus 34 days in a 23°C environment. Each block represents the response of eight squirrels.

It can be seen that significantly higher survival occurred among squirrels irradiated while torpid as compared to those irradiated while active. No significant differences were noted in percent survival between the various groups irradiated while torpid. After removal to a 23°C environment, squirrels continued to die, but at a lower rate than during the first 8 weeks. However, three squirrels irradiated while

TABLE I

PERCENT SURVIVAL OF IRRADIATED (1250 RADS, ^{60}Co) GROUND
SQUIRRELS WITH IMPLANTED TRANSMITTERS AND OF
CONTROL GROUND SQUIRRELS WITH IMPLANTED TRANS-
MITTERS AFTER 8 WEEKS IN RESPECTIVE TEMPERATURE
ENVIRONMENTS (UPPER PORTIONS) AND AFTER 90 DAYS POST-
IRRADIATION (LOWER PORTIONS). DETAILS ARE GIVEN IN
THE TEXT

Postirrad. *Envir. temp.* *(°C)*	*Percent survival* *Irradiation body temperature (°C)*			
	5	*13*	*37*	*Controls*
5	63	63	25	100
	50	63	25	
13	75	63	25	100
	75	50	13	
23	75	75	0	100
	63	50	0	

active and maintained in the cold environments survived 90 days whereas all those maintained at 23°C died. There were no deaths among the 20 control squirrels.

At the end of 8 weeks 31 transmitters were still functioning properly. During the 8-week period 39 squirrels had died. Among these, 27 had properly functioning transmitters at the time they were removed from the carcasses. Twenty-four transmitters gave practically no useful information; 53 transmitters gave data on the activity of irradiated squirrels and 15 transmitters gave data about control squirrels.
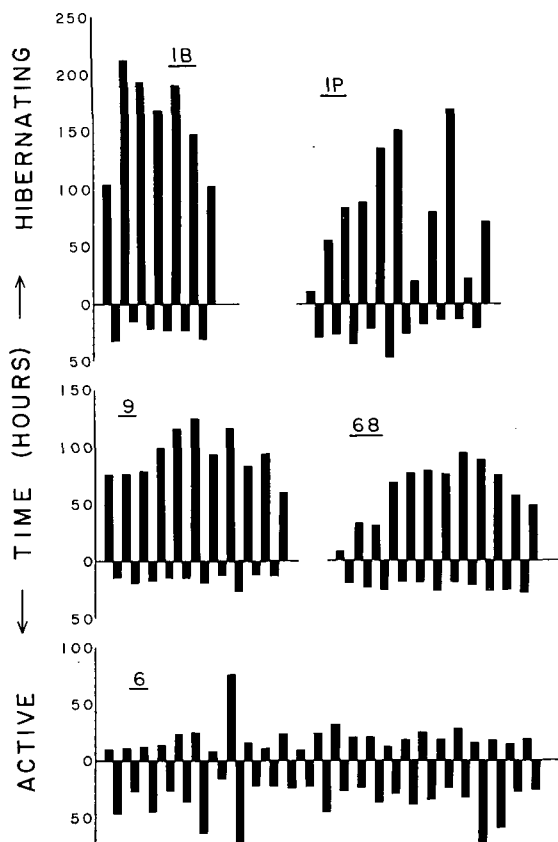

FIG. 1. Individual hibernation activity cycles of irradiated ground squirrels obtained from telemetry data during an 8-week period in temperature environments of 5°C (9, 1B, 1P), 13°C (68) and 23°C (6). Periods given do not include time required to enter into or arouse from hibernation. Each solid bar above the zero line represents a period of hibernation, each solid bar below the zero line represents a period of activity (normothermia). Abscissas are time scales, normalized to hibernation activity cycles (see text also).

Thus from 58 properly functioning transmitters and ten transmitters that gave information part of the time, information on metabolic states was obtained (see also Fig. 2).

Examples of hibernation activity cycles recorded are shown in Fig. 1. The abscissa begins at the first monitored hibernation period and progresses through hibernation activity cycles until the squirrels were removed from their receiving stations. Hibernation periods of monitored squirrels varied from 5 h to 12 days. In most instances a given squirrel had relatively constant hibernation periods. Among the squirrels that died, during 90 days of observation, the longest period of hibernation was 5 days. Three of the 15 control animals monitored remained active over the entire period of observation. Hibernation periods of the others varied from 11 hr to 10 days.

Among irradiated squirrels maintained at the lower environment temperatures, more animals hibernated and hibernation periods were longer. Of 14 squirrels that hibernated in the 5°C environment, eight had average hibernation periods over 100 h. The nine irradiated squirrels hibernating in the 13°C environment had average hibernation periods between 40 and 80 h. Only five irradiated squirrels hibernated at 23°C, with average periods of less than 35 h. Among squirrels that hibernated, most took between 2 and 5 days to begin hibernating; seven took more than 10 days.

Fourteen of 20 monitored squirrels irradiated while active remained active until death. The three surviving squirrels irradiated while active went through hibernat-

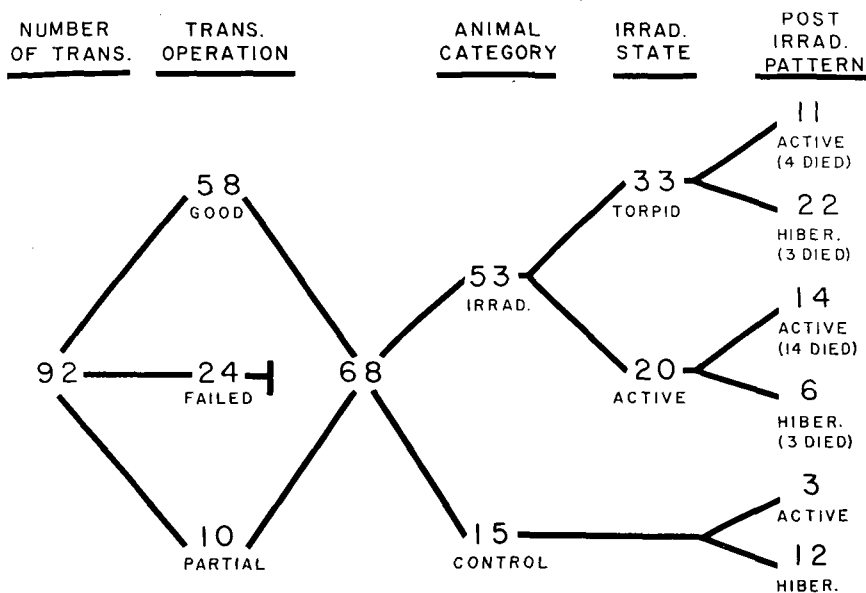| NUMBER OF TRANS. | TRANS. OPERATION | ANIMAL CATEGORY | IRRAD. STATE | POST IRRAD. PATTERN |

FIG. 2. Flow chart illustrating the grouping and numbers of transmitters. The last column shows a tabulation of data obtained with the transmitters.

ing cycles. Among monitored squirrels irradiated while hibernating, 11 were active during most of the postirradiation period or until death (four in this category); 22 squirrels underwent hibernation cycles during most of the observation period or until death (three in this category) (see Fig. 2).

DISCUSSION

The protocol of this experiment was similar to that of an earlier experiment (5) except for the telemetry aspect. The results, both 100 % survival of controls and similarity in percent survival of the irradiated groups of squirrels to that in the previous experiment, indicate that transmitter implantation had little or no effect on mortality figures. A number of squirrels implanted with nonfunctioning transmitters and not used in this experiment were sacrificed at various times to study tissue reaction to the transmitters. Almost all of these squirrels had a thin sheath of connective tissue surrounding the transmitter; a few had vascular beds developed within this covering. No anatomical abnormalities were seen in any of the visceral organs.

It was the purpose of the present investigation to determine if there was a relationship between postirradiation hibernation and survival of irradiated squirrels. Such a relationship was indicated by the fact that among squirrels irradiated while torpid, 85 % that hibernated survived as compared to 64 % that remained active. Also, the only three surviving squirrels among those irradiated while active went through hibernation cycles. Increased survival because of postirradiation hibernation has been suggested by others (4). It is reasonable to conclude from the present work and from previous studies (3, 5, 7) that such an increase in survival would be in addition to the marked increase in survival due to torpidity during irradiation.

It appeared that there was no difference in response caused by the three different postirradiation environments; even though at warmer environmental temperatures, average hibernation periods were shorter. From a comparative point of view, our results of hibernation activity cycles in *C. tridecemlineatus*, showing shorter cycles at the higher hibernating temperatures, were in keeping with the extensive observations of *C. lateralis* obtained by Twente and Twente (1965).

Although experimental protocol did not permit definitive statements concerning possible effects of irradiation on hibernation ability of ground squirrels, there was an indication that an effect exists. Eighty percent of monitored control squirrels hibernated, whereas 67 % of monitored squirrels irradiated while torpid hibernated and only 30 % of monitored squirrels irradiated while active hibernated.

It was disappointing to have had some transmitters malfunction. Causes for this are discussed in another paper (6). Earlier tests showed that these transmitters functioned properly after ^{60}Co exposure doses as high as 75 kR. In general, the operation of the telemetry system was considered satisfactory.

Conclusions obtained from this investigation are that ground squirrels have a degree of radio-protection due to torpidity during irradiation. Further limited

increase in survival rate appears to be related to hibernation after irradiation, at least at a radiation dose of 1250 rads. However, a cause and effect relationship between postirradiation hibernation and increased survival remains undefined. Postirradiation environmental temperatures from 5 to 23°C had no effect on survival rate.

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Synergistic role of temperature and exposure to ionizing radiation[1]

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SYNERGISTIC EFFECTS OF temperature and ionizing radiation in mammalian species have been investigated from several approaches. Combinations of exposures to low temperature and X-radiation were examined in rats by Kimeldorf and Newsom (10) and by Ghys (5), and in mice by Smith et al. (16). The effects of high temperatures and radiation response have been tested in the hamster (2). Except for the results obtained by Ghys (5), combinations of exposure to low or high temperatures and irradiation usually result in an increase in percent mortality and lower LD_{50}'s.

Another approach to studies of temperature and radiation combinations is in modification of temperature regulation to the point of metabolic depression, namely, hibernation and hypothermia. This subject was reviewed a short time ago by Musacchia and Barr (12) and they summarized a series of experiments which clearly demonstrated that hibernating ground squirrels *Citellus tridecemlineatus* and hypothermic hamsters *Mesocricetus auratus* showed increased levels of radioresistance. This was reported in terms of higher LD_{50}'s, higher percent survival, and increased dose reduction factors. Comparisons were made between active normothermic (controls) and hibernating or hypothermic animals at various radiation exposures (e. g., 500–20,000 rads).

Several questions have evolved from points of contradiction in studies of hibernation and radioresistance. Smith (15) and later Jaroslow et al. (8) hold that radioresistance is evident during hibernation and on arousal radiation pathology is comparable to that in irradiated nonhibernating animals, whereas Barr and Musacchia (1, 11) provide evidence that irradiation during hibernation shows lasting effects even after arousal. Weiss (19) and Hornsey (7) provide evidence that with extreme low levels of hypothermia hypoxia is the protective agent. Volkert and Musacchia (18) showed that at hypothermic levels of body temperature 7 C the hamster is only "mildly" hypoxic and yet shows significant levels of increased radioresistance (14). Thus limited tissue oxygen

appears only a partial answer to increased radioresistance. Last, a question should be raised about the effects of cold per se. Since both hibernation and hypothermia are mediated through cold exposure, is there any effect which can be credited directly to cold exposure? At this point it may be well to recall also the unique report of Ghys (5) which claims increased radioresistance following exposure to low ambient temperature.

The objectives of the present research were to answer some of the questions cited above and to reduce some of the ambiguities found in this area of research. To this end we have utilized one uniform radiation source and narrowed experimental subjects to one species. The hamster was selected as the mammal of choice because it can be made to hibernate, it can undergo experimental hypothermia, and it readily acclimates to low temperature. Therefore it meets the three experimental criteria already referred to in the literature and in previous experiments in our laboratory.

MATERIALS AND METHODS

Experimental animals. "Syrian" or "golden" hamsters *Mesocricetus auratus* were taken from a closed colony established in our laboratory in the fall of 1966. Animals, both males and females, ranged in age from 3 to 5 months and body weights ranged from 100 to 130 g. Whenever possible, in about 70% of the experiments, siblings were used as control and experimental subjects.

Hibernation. Hamsters were induced to hibernate in a darkened cold room at ambient temperature 5 C. Each animal was individually housed in a plastic cage, given cedar wood shavings for bedding and nesting purposes and an ad libitum diet of Wayne Lab Blox and water. Hibernation occurred after lengthy periods of cold exposure, usually 6–8 weeks. During hibernation, body temperatures reached 6–7 C. Irradiation exposures were done without interruption to hibernating dormancy and those animals were then permitted to awaken. Each animal was housed individually and maintained in an animal stock room with a light cycle of 12 dark and 12 light hours, and an ad libitum diet as before hibernation. Each animal was checked at least twice daily.

Hypothermia—long term. Hamsters were made hypothermic by the helium-cold method (4, 14). Briefly described, the hamster is placed in a glass container with a constant flow (150–200 ml/min) of helox (helium + oxygen, 80:20) at 0 C. After several hours the animal becomes progressively hypothermic; on reaching body temperature about 10 C the hamster is removed from the helox and left in the ambient temperature 0 C cold room until body temperature reaches 7 C. At this point the animal is moved to another cold chamber set at 7 C. Hamsters which were hypothermic for periods of 16–20 hours at body temperature 7 C were irradiated, again without interruption of hypothermic depression. Following irradiation each animal was permitted to revive, was individually housed and maintained comparable to irradiated hibernating and control animals.

Cold acclimation. Each animal was housed individually and in a manner identical to those described above. Cold exposures were done in environmental rooms at ambient temperature 7 C for periods of 2 or more weeks. Animals were irradiated and either returned to the cold room or brought to the animal holding room at ambient temperature 22–23 C. Other hamsters were taken from standard stocks at ambient temperature 22–23 C, irradiated and then exposed to cold, ambient temperature 7 C. In all instances animals were maintained singly and under identical caging conditions as the hibernating and hypothermic animal.

Irradiation exposures. All animals were given doses of whole-body ionizing radiation at a dose rate of approximately 210 rads/min. Irradiations were accomplished using a Gamma 12 (U. S. Nuclear) ^{60}Co irradiator. Details were published earlier (1).

Catecholamine analysis. After body temperature of hamsters was reduced to 7 C, the animals were sacrificed by cervical dislocation. The hearts were excised, atrial tissues were removed, and after blotting, the ventricles were frozen with dry ice. The same protocol was followed on a control group sacrificed at normal body temperature. The ventricles were analyzed for catecholamines according to the method of Crout (3) adapted to the AutoAnalyzer by Jellinek et al. (9).

RESULTS AND DISCUSSION

Irradiation and depressed metabolism. Depressed metabolic states induced by cold, namely, hibernation and hypothermia, have been credited with providing increased radioresistance in mammals (1, 7, 11, 14, 19). Comparative studies of radioresistance within the same species in different depressed metabolic states have not been done. Results showed that the hibernating hamster has approximately the same level of radioresistance as its hypothermic counterpart. After 1,000 rads of ^{60}Co radiation, comparative resistance to this exposure in terms of survival for hamsters were: long-term hypothermic 90%, hibernating 85%, and normothermic 10% (Fig. 1). The percent survival versus dose curve as done for hypothermic hamsters (14) is being developed for the hibernating hamster.

One potential mechanism responsible for increased radioresistance has been studied, that is, the role of hypoxemia in hypothermia and in hibernation. It was observed (18) that there was a decreased venous Po_2 in hypothermic hamsters (body temperature 6 C) even though arterial blood was completely saturated. The venous Po_2 in hypothermic hamsters was 9.1 ± 1.1 mm Hg compared to 37.6 ± 4.5 mm Hg found in normothermic hamsters. Similar results were found in hibernating ground squirrels (13). During hibernation (body temperature 6 C) of these animals the venous Po_2 was 6.3 ± 1.2 mm Hg compared with a value of 27.9 ± 2.2 mm Hg in the normothermic animal. These results provide evidence of a limited protective "oxygen effect" which may be related to improved radioresistance in hibernation and hypothermia.

Catecholamines have been shown to increase radioresistance in a variety of mammalian species (6, 17). We have suggested that naturally occurring modifications in tissue catecholamines in depressed metabolic states might provide insight into the mechanism of increased radioresistance (12).

In a current experiment, it was found that norepinephrine and epinephrine levels were reduced in the ventricles of hypothermic hamsters compared with control normothermic hamsters. The difference is significant at the 0.05 level (Table 1).

If the reduced levels of catecholamines in the heart are indicative of changes in tissue catecholamines in other tissues during hypothermia, this suggests that the radio protection seen in hypothermic hamsters is not due
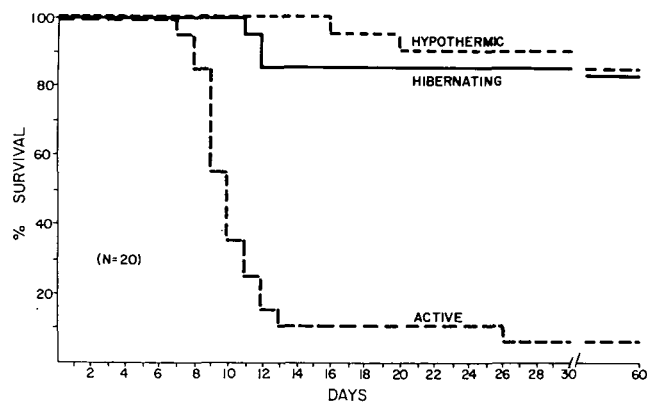

FIG. 1. A comparison of radioresistance in depressed metabolic states in the hamster following whole-body exposures to high lethal levels of ionizing radiation.

TABLE 1. *Catecholamine content in ventricles of hypothermic, body temperature 7 C, and normothermic, body temperature 37 C, hamsters*

	Control	Hypothermia
Norepinephrine, $\mu g/g$ wet weight	1.38±.06	0.459±.054
Epinephrine, $\mu g/g$ wet weight	0.038±.005	0.020±.003

to high levels of tissue catecholamines. However, it is still possible that the release of catecholamines may contribute to radioprotection during the induction of hypothermia or with the recovery from hypothermia. The role of catecholamines in radioresistance in depressed metabolism in hamsters requires considerable investigation.

Irradiation and cold exposure. The experiments of Kimeldorf and Newsom (10) showed that rats (Sprague-Dawley) exposed to cold (ambient temperature 6 C) following various high levels of radiation had lowered survival rates. For example, from their data it appears that the $LD_{50/30}$ for rats maintained at ambient temperature 6 C is between 500 and 550 rads whereas for rats maintained at ambient temperature 23 C it is between 550 and 600 rads. They suggest that "increased metabolic activity may be an important factor" in acceleration of radiation death.

A most interesting report concerning the response of rats (Sprague-Dawley) to low temperature exposure and radiation is that of Ghys (5). He reports that rats which are chronically irradiated at low dose rates after being cold acclimatized at ambient temperature 5 ± 1 C for 1 month show an increased level of radioresistance. The radioprotective "factor" is about 1.6. Furthermore, he claims that the protective action of the cold acclimatization lasts for at least 1 week after the animals are removed from the cold.

One of the earliest reports dealing with the effect of environmental temperature and X-radiation was that of Smith et al. (16). They showed that with mice (males, NIH stock) cold exposure following irradiation had a detrimental effect and resistance to lethal levels of X-radiation was lowered.

To date, with the limited data available one might speculate that cold exposure following irradiation with high levels, i. e., close to the LD_{50}, is detrimental. On the other hand, cold exposure prior to irradiation might prove beneficial in providing some effective increase in radioresistance. It is of note that none of the investigations herein reviewed offer any substantive explanation for decreased or increased radiosensitivity as related to cold exposure before or after irradiation. At best one finds the speculation that there were alterations in total metabolic activity as reflected in ordinary mammalian response to cold. In our opinion, with cold exposure following radiation the animal is faced initially with conditions of thermoregulatory modification. In the rat it is known that physiological adjustment to cold requires several days with increased energy costs. Such conditions coupled with the radiation-induced effects which are evident soon after exposures could explain the findings of Kimeldorf and Newsom (10) that the most deleterious radiation effects were seen during the first 2 weeks of postirradiation cold exposure.

Equipped with the information that synergistic effects of cold and radiation, particularly when irradiation is followed by cold exposure, are potentially deleterious and that cold exposure prior to irradiation might afford some increased radioresistance, we designed a series of experiments to test various combinations of cold and radiation exposures. In these experiments hamsters were exposed to cold before or after acute irradiation. Control animals were maintained at room temperature before and after irradiation. Several different levels of radiation exposures were used at a dose rate of about 210 rads/min.

The initial series of experiments involved doses well over the LD_{50} levels, purposefully to determine if cold effects could be noted in areas where both gastrointestinal and hematopoietic syndromes are affected. Although a variety of acclimation periods were used, only data for 3- and 8-week exposures are reported. There were no differences in responses (with 1,000, 1,100, and 1,200 rads) at any acclimation periods (Fig. 2).

It is evident that with 1,200 rads the radiation effects supersede any pre- or posttemperature effects. The cascading death in these animals occurs between *days 7* and *9*. At the next lower doses (1,000–1,100 rads) the predominant rate of deaths is between *days 8* and *14*. Again, there are no significant changes in terms of cold acclimatization or postirradiation exposure to cold.

A series of experiments with exposures of 850 and 900 rads, i. e., close to the $LD_{50/90}$ for these animals, 875 rads (14), provides data which show that cold exposure before irradiation may afford a slight degree of radioprotection (Fig. 3). However, if protection relatable to cold exposure at these lower dose levels is provided, it is certainly minimal. Thus, the data indicate that acclimation of hamsters to cold ambient temperature 7 C prior to acute exposure to ^{60}Co γ-rays provides little or no protection. In addition, the combined insult of cold exposure following irradiation appears to be most pronounced and results in the lowest levels of survival (Fig. 3). These results with hamsters tend to agree with an earlier report of lethality in rats during postirradiation cold exposure (10).

Comparative aspects of radioresistance. An evaluation of comparative levels of radioresistance relatable to cold exposure and cold-induced depressed metabolic states was made in terms of dose reduction factors (DRF = LD_{50} experimental animals/ LD_{50} control animals) and presented in Table 2. It is evident that hypothermic and hibernating hamsters have comparable dose reduction factor values. They are slightly less than those of hibernating ground squirrels. The most significant dose reduction factor is that reported for hypothermic mice at body temperature 0 C (7). These mice, however, are severely hypoxic and differ greatly from the helium-cold hypothermic hamster which is only mildly hypoxic. The hypothermic hamster is a viable animal, despite the considerable reduction in physiological activities, it can still rewarm and become active with no greater stimulus than exposure to ambient room temperature 22–24 C. The dose reduction factor 1.6 for the cold-acclimated rats indicates a level of radioprotection which is better than that afforded by hibernation and hypothermia and less than that provided by hypoxia. However, it must be
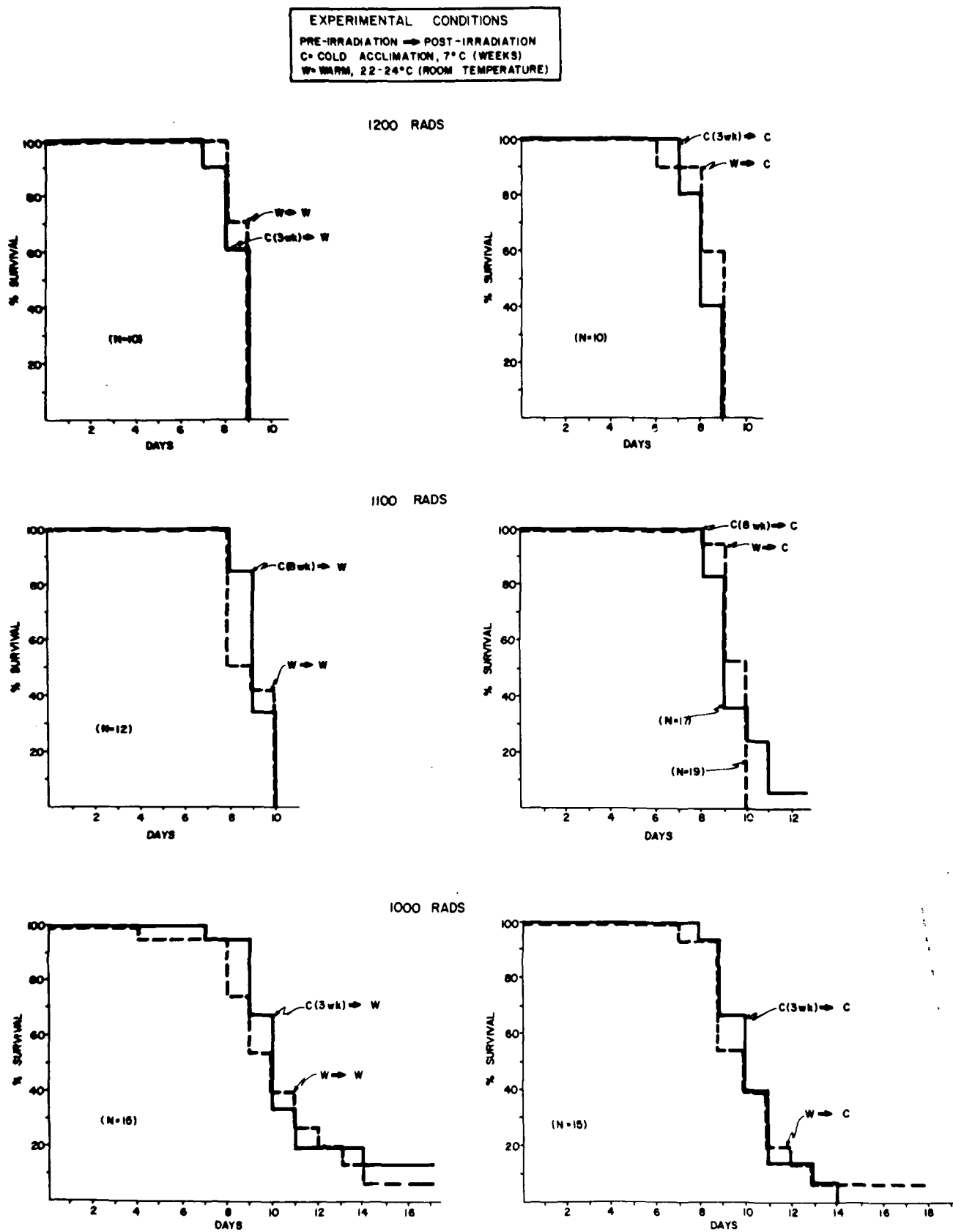
FIG. 2. Cold exposure and radiation responses with whole-body exposures to high lethal levels of ionizing radiation.
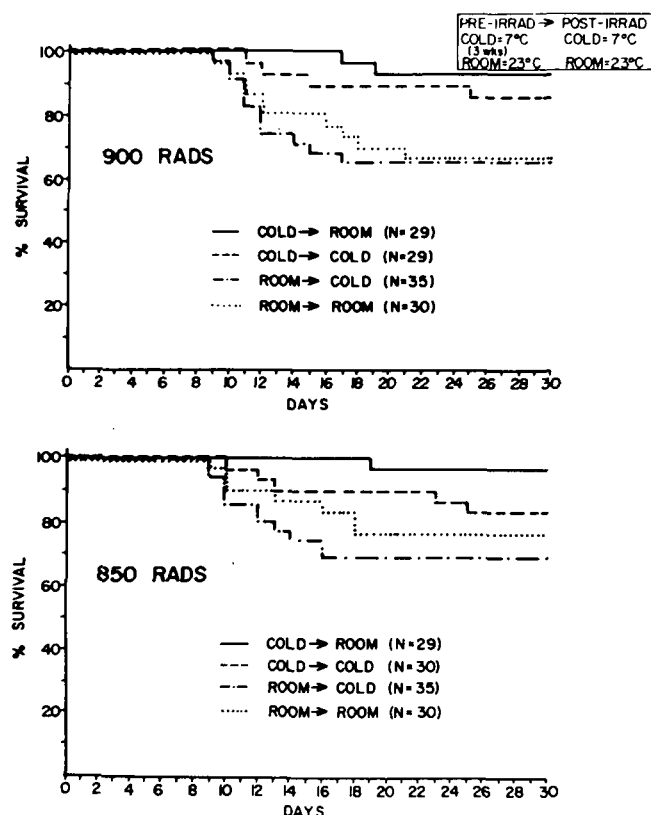
FIG. 3. Cold exposure and radiation responses with whole-body exposures to levels of ionizing radiation close to LD_{50} (875 rads) for hamster (12).

noted that Ghys' (5) exposures were chronic rather than acute. In all other instances herein reported radiation exposures have been acute. There is as yet no adequate explanation for Ghys' results.

The results of cold exposure experiments leave a wide area for conjecture. Briefly, in our opinion there is still lacking a comprehensive experiment that could disclose whether or not radiation effects are mitigated or exaggerated with exposure to cold before or after irradiation.

SUMMARY AND CONCLUSIONS

Increased levels of radioresistance are evident in animals irradiated while in states of depressed metabolism and then permitted to arouse. This conclusion is based on published information in which hibernating ground squirrels *Citellus tridecemlineatus* were used and the experimental results herein reported for hibernating and hypothermic hamsters *Mesocricetus auratus*. The radiation responses have been assessed by routine radiobiological measures: percent survival, mean survival times, calculated LD_{50}'s and dose reduction factors. Although the physiological mechanism(s) responsible for this increased radioresistance is not known, there is an initial implication of the occurrence of a mild level of tissue hypoxia. It is of note, however, that the level of metabolic depression in hibernation and helium-cold hypothermia is "physiological." This is intended to mean that these animals easily revive, become normothermic and resume

TABLE 2. *Increases in resistance to lethal levels of ionizing radiation in animals with cold-induced depressed metabolic states during irradiation or acclimated to cold prior to irradiation*

Animals	Environmental Conditions	Dose Reduction Factor	References
Mice	Hypothermic (T_b 0–1 C)	2.8	(7)
Rats	Acclimated to cold (T_a 5 C) for 1 month	1.6	(5)
Ground squirrels	Hibernating (T_b 6 C)	1.4–1.6	(1)
Hamsters	Hypothermic (T_b 7 C) (short-term)[a]	1.2–1.3	(13)
Hamsters	Hibernating (T_b 7 C)	1.2–1.3[b]	
Hamsters	Hypothermic (T_b 7 C) (long-term)[a]	1.3[b]	
Hamsters	Acclimated to cold (T_a 7 C) 3–8 weeks	1[b]	

T_a, ambient temperature; T_b, body temperature. [a] Short-term = hypothermic 30 min or less, long-term = hypothermic 16–18 hours. [b] Based on experiments herein reported.

biological and behavioral activities showing no evidence of having undergone metabolic depression. These approaches may provide a means for explanation of mechanisms of radioprotection heretofore restricted by levels of hypothermia which, for all intents, left animals in a moribund state.

The role of tissue catecholamines and catecholamine metabolism in depressed metabolic states remains to be investigated. The initial results herein presented suggest a fruitful area for radiobiology and low temperature physiology.

A lack of protective effect is evident where combinations of lethal levels of ionizing radiation and either pre- or postradiation cold exposures are used. Where irradiations close to LD_{50}'s are concerned, there is some indication that prior cold acclimation improves radioresistance in the hamster. However, cold exposure after irradiation appears to be synergistically detrimental. The effects of low levels (much less than LD_{50}'s) of radiation and cold exposures are wholly unexplored. The most significant question raised at this time is the role of increased catecholamine sensitivity in cold exposed animals and the known increase in radioresistance with administration of catecholamines. Although it may appear highly speculative, it seems reasonable to suggest that if, with additional experiments, significant levels of increased radioresistance become evident, it would be well to focus attention on bio-amine metabolism as the common denominator of cold exposure and radioresistance.

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B. Titles of Papers Presented and Abstracts.

1. Helium - Oxygen Induced Hypothermia: Effects on Hamsters Hepatic Mitochondria. M. E. Thompson, M. L. Vorbeck, F. V. Lucas, X. J. Musacchia, and A. P. Martin. Fed. Proc. 31:641. 1972.

2. In Vitro Oxidation of Radioactive Substrates by Tissues from Hamsters Made Hypothermic with Helium and Cold. Cecil Entenman, X. J. Musacchia and John Walsh. Fed. Proc. 31:826. 1972.

3. The Role of Carbohydrate in Hypothermia. Garth Resch, Gary Anderson and X. J. Musacchia. Proc. Mo. Acad. Sci. (in press) 1972.

4. Identification of Factors Pertinent to Increase Survival in Helium-Cold Hypothermic Hamsters. X. J. Musacchia, Wynn Volkert, Garth Resch and G. L. Anderson. The Physiologist 15:223. 1972.

HELIUM-OXYGEN INDUCED HYPOTHERMIA: EFFECT ON HAMSTER HEPATIC
MITOCHONDRIA. M. E. Thompson*, M. L. Vorbeck*, F. V. Lucas,
X. J. Musacchia*, and A. P. Martin*. Univ. of Missouri Sch. of
Med., Columbia, Mo. 65201

Hypothermia was induced in hamsters (M. auratus) using a
mixture of 80% helium and 20% oxygen (helox), and cold ex-
posure. Animals were exposed to helox and cold until body
temperature dropped to 7-10°C. Animals were then removed from
the helox and maintained at 7°C for an average of 20 or 44
hours. Mitochondria were isolated using standard differential
centrifugation techniques. Respiratory control ratios and
activities of oxidative enzymes associated with electron trans-
port (cytochrome c oxidase, succinoxidase, succinic dehydrogen-
ase) were determined. Respiratory control ratios for mitochon-
dria isolated from 20-hour hypothermic hamsters were similar to
the mitochondria isolated from control hamsters (3.0), whereas
those isolated from 44-hour hypothermic hamsters showed a sig-
nificant decrease in the degree of coupling (2.7) compared to
their corresponding control (3.1). Little difference in the
activities of oxidative enzymes associated with electron trans-
port was observed for the hypothermic as compared to the normo-
thermic hamster. Sedimentation velocity studies have shown
distinctive differences in the banding pattern of mitochondria
isolated from livers of hypothermic and normothermic control
hamsters. Under identical centrifugal conditions the sedimen-
tation velocity for mitochondria isolated from hypothermic ham-
sters was shown to be less than that for paired control. This
shift in banding pattern suggests a change in mitochondrial
size as a consequence of the hypothermic state.

Environment and Exercise

IN VITRO OXIDATION OF RADIOACTIVE SUBSTRATES BY
TISSUES FROM HAMSTERS MADE HYPOTHERMIC WITH HELIUM
AND COLD. Cecil Entenman, X.J. Musacchia and John
Walsh*. Inst. Lipid Res., Berkeley, Calif. 94702,
Physiol. Dept. and Space Sci. Res. Ctr., Univ. Mo.,
Columbia, Mo. 65201
    In order to measure the influence of helium-cold
hypothermia on various metabolic pathways operative
at body temperature 7C, tissue slices from 1-hr
hypothermic hamsters (T_{re} 7C) were incubated in
vitro, 2 hr in Krebs-Ringers bicarbonate buffer with
20 mM amounts of various substrates. $^{14}CO_2$ was col-
lected during incubations and nanomoles of each sub-
strate oxidized per gm tissue in 2 hr was calculated.
Incubations were carried out at 7 and 37C. Controls
were sibling, fasted, normothermic hamsters. Tis-
sues used were adipose (A), brown fat (BF), diaphram
(D), heart (H), kidney (K), liver (L), and small
intestine (SI). Oxidation of all substrates was
less at 7 than at 37C, but glutamate was less temp-
erature sensitive than other substrates in all tis-
sues. In all tissues except A, pyruvate and gluta-
mate were comparably oxidized. K and SI had highest
oxidations but were also the most temperature sensi-
tive. Few differences were noted between normal and
hypothermic tissues incubated at 7 and 37C, showing
the value of the helium-cold method. (Supported by
NASA Grants NGR 05-035-005 and NGR 26-004-021.)

ROLE OF CARBOHYDRATE IN HYPOTHERMIC SURVIVAL

Garth Resch, Gary Anderson, X.J. Musacchia

Space Sciences Research Center and Department of Physiology

University of Missouri

For several years we have been interested in problems concerned with survival in hypothermia, where this depressed metabolic state is induced by exposure to 80% helium, 20% oxygen and low ambient temperature, ca. T_a 0°C. Earlier work in this laboratory indicated that the availability of endogenous carbohydrate to the hypothermic hamster was an important factor in hypothermic survival. In recent experiments it was found that blood glucose concentration followed a predictable pattern through the course of survival in hypothermia, and that low blood glucose, ca. 10 mg%, correlate with termination of hypothermic survival and subsequent death.

The present work extends these studies. Golden Hamsters, Mesocricetus auratus, weighing 100 to 130 gm were given 0.1 ml of 200 mg% exogenous glucose (intravenously) at approximately 3-4 hour intervals during the hypothermic period. Experimental and control subjects were induced into hypothermia simultaneously. Animals at T_b 7°C were maintained in cold rooms at T_a 7°C. In hamsters with glucose administration, hypothermic survival was enhanced approximately fivefold as compared with control animals which were given equivalent amounts of 0.9% saline. This research was supported by SSRC and grants USPH ES-114-03 and NASA NGL 26-004-021S6.

IDENTIFICATION OF FACTORS PERTINENT TO INCREASED SURVIVAL IN
HELIUM-COLD HYPOTHERMIC HAMSTERS. <u>X.J. Musacchia, Wynn
Volkert</u>*, Garth Resch* and G.L. Anderson*. Physiol. Dept.
and Space Sci. Res. Ctr., Univ. Mo., Columbia, Mo. 65201

Two features have been identified as relevant to survival
in hypothermic (T_{re}7C) hamsters: A) shortened induction time
and B) depletion of carbohydrate metabolites. Induction time
is shortened in two ways: by prior heat acclimation, and by
adding 2.5% halothane into the helium:oxygen (80:20) helox
mixture at T_a0C. Induction times, from normothermia T_{re}37C
to hypothermia T_{re}7C, were as follows: "routine" 80:20
helox, 80:20 helox + halothane, 2 wk heat acclimated
(T_a34C) + "routine" 80:20 helox, and 2 wk heat acclimated
(T_a34C) + 80:20 helox + halothane, respectively 5.8, 1.6, 3,
and 1.5 hrs. In the same order, survival times in hours at
T_{re}7C were 27, 106, 59, and 103. Reduced induction times are
relatable to increased survival in hypothermia. In addition,
a combination of helox and halothane is an improved method
for inducing hypothermia. Both liver glycogen and blood glu-
cose levels are affected by hypothermia and are relatable to
survival. Liver glycogen falls from 69.2 to as low as 2.5
mg/g w.w. With induction of hypothermia, blood glucose falls
from 100-110 to @10 mg% when death ensues at 24-27 hr. There
is a sparing action with rapid induction (helox + halothane).
With replenishment of blood glucose via a carotid cannula,
200 mgs/ml every 4 hrs, blood level is sustained at 30-45
mg% and survival increased to 2-3 days. (Supported by NASA,
NGR 26-004-021.)